College Misericordia Library
Dallas, Pennsylvania

College Misericordia Library
Dallas, Pennsylvania

College Misericordia

Digitized by the Internet Archive
in 2026

https://archive.org/details/technicalmycolog0002drfr

TECHNICAL MYCOLOGY

VOL. II.

NET BOOK.—This book is supplied to the Trade on terms which will not allow of Discount to the Public.

CHARLES GRIFFIN & COMPANY, LTD.

STANDARD WORKS.

BREWING: The Principles and Practice of. For the Use of Students and Practical Men. By WALTER J. SYKES, M.D. Third Edition revised by ARTHUR R. LING, Editor of the "Journal of the Institute of Brewing." In large 8vo, handsome cloth, price 21s. net.

"A volume of Brewing Science, which has long been awaited. . . . One of THE MOST COMPLETE IN CONTENTS and NOVEL IN ARRANGEMENT that has yet been published. . . . Will command a large sale."—*The Brewers' Journal.*

"The appearance of a work such as this serves to remind us of the ENORMOUSLY RAPID ADVANCES made in our knowledge of the Scientific Principles underlying the Brewing Processes. . . . Dr. Sykes' work will undoubtedly be of the GREATEST ASSISTANCE, not merely to Brewers, but to all Chemists and Biologists interested in the Problems which the Fermentation Industries present."—*The Analyst.*

AN ELEMENTARY TEXT-BOOK OF BIOLOGY. By J. R. AINSWORTH DAVIS, B.A., Professor of Biology, University College, Aberystwyth. SECOND EDITION. In Two Volumes. Sold separately.

I. **Vegetable Morphology and Physiology.**
With Complete Index-Glossary and 128 Illustrations. 8s. 6d.

II. **Animal Morphology and Physiology.**
With Complete Index-Glossary and 108 Illustrations. 10s. 6d.

"Certainly THE BEST 'BIOLOGY' with which we are acquainted."—*British Medical Journal.*

WORKS BY A. WYNTER BLYTH, M.R.C.S., F.C.S.,
Barrister-at-Law, Public Analyst for the County of Devon, and Medical Officer of Health for St. Marylebone,
AND M. WYNTER BLYTH, B.A., B.Sc., F.C.S.

FOODS: Their Composition and Analysis, with the Detection of Adulterations. In large 8vo, with Elaborate Tables, Diagrams, and Plates. Handsome cloth. SIXTH EDITION. Thoroughly revised. Price 21s.
"Simply INDISPENSABLE in the Analyst's laboratory."—*The Lancet.*

POISONS: Their Effects and Detection. FOURTH EDITION. In large 8vo, cloth, with Tables and Illustrations, price 21s. net.
"Undoubtedly THE MOST COMPLETE WORK on Toxicology in our language."—*The Analyst* (*on the Third Edition*).

FERMENTS, AND THEIR ACTIONS: a Text-Book on the Chemistry and Physics of Fermentative Changes. By CARL OPPENHEIMER, Ph.D., M.D. Translated by C. A. MITCHELL, B.A., F.I.C. Cloth, 7s. 6d. net.
"Such a veritable *multum in parvo* has never yet appeared."—*Brewers' Journal.*

MICRO-ORGANISMS & FERMENTATION. By ALFRED JÖRGENSEN. Translated by SAMUEL H. DAVIES, M.Sc. FIFTH EDITION, Revised Throughout. With 101 Illustrations. In Cloth, 15s. net.
CONTENTS: Microscopical and Physiological Investigations.—Investigation of Fermentation in Air and Water.—Bacteria.—Moulds.—Yeasts.—Culture.—BIBLIOGRAPHY.—INDEX.

LONDON: CHARLES GRIFFIN & CO. LTD., EXETER STREET, STRAND

TECHNICAL MYCOLOGY

THE UTILIZATION OF MICRO-ORGANISMS IN THE ARTS AND MANUFACTURES

A PRACTICAL HANDBOOK ON

FERMENTATION AND FERMENTATIVE PROCESSES FOR THE USE OF BREWERS AND DISTILLERS, ANALYSTS, TECHNICAL AND AGRICULTURAL CHEMISTS, PHARMACISTS, AND ALL INTERESTED IN THE INDUSTRIES DEPENDENT ON FERMENTATION

BY

DR. FRANZ LAFAR,

Professor of Fermentation-Physiology and Bacteriology in the Imperial Technical High School, Vienna

Translated by CHARLES T. C. SALTER

VOL. II.—EUMYCETIC FERMENTATION

WITH ONE HUNDRED AND EIGHTEEN FIGURES IN THE TEXT

LONDON

CHARLES GRIFFIN AND COMPANY, LIMITED

EXETER STREET, STRAND

1911

[*All rights reserved*]

PRINTED BY
BALLANTYNE & COMPANY LTD
AT THE BALLANTYNE PRESS
TAVISTOCK STREET COVENT GARDEN
LONDON

TABLE OF CONTENTS.

DIVISION II.

EUMYCETIC FERMENTATION.

Section X.—Rudiments of the General Morphology and Physiology of the Eumycetes.

Chapter XXXIX.—General Morphology.

	PAGE		PAGE
§ 217. Articulation of the Thallus .	1	§ 222. Fructification by Conidia . .	15
§ 218. The Typical Mycelium . .	3	§ 223. The Formation of Oidia and Gemmæ	18
§ 219. The Gemmating Mycelium .	7	§ 224. The Germination of Spores. Their Tenacity of Life . .	20
§ 220. Fructification by Sporangia .	11		
§ 221. Fructification by Zygospores .	13		

Chapter XL.—Chemical Composition of the Cell Membrane of Eumycetes.

§ 225. Cellulose	24
§ 226. Chitin	26
§ 227. Hemicelluloses and other Carbohydrates	28

Chapter XLI.—Mineral Nutrient Materials.

§ 228. Alkalis	31
§ 229. Metals of the Alkaline Earths .	32
§ 230. Elements of the Iron Group .	34
§ 231. Sulphur, Selenium, Silicon, Phosphorus, Arsenic . .	36

Chapter XLII.—Stimulative Influences—General Remarks on the Enzymes of Eumycetes.

§ 232. Influence of Light on the Development of the Eumycetes	39
§ 233. Chemotropism — General Remarks on the Enzymes of Eumycetes	43

Section XI.—Fermentation by Zygomycetes.

Chapter XLIII.—Morphology and Systematology of the Mucors.

§ 234. Subdivision of the Order of Zygomycetes	48
§ 235. Subdivision of the Mucor Family	49
§ 236. The Genus Mucor . . .	51
§ 237. Rhizopeæ	53

6178.

CHAPTER XLIV.—FERMENTATION BY MUCORS.

§ 238. Intramolecular Respiration and Alcoholic Fermentation . 57
§ 239. Mucor Fermentations . . 59

CHAPTER XLV.—THE USE OF MUCOREÆ IN THE SPIRIT INDUSTRY.

§ 240. Mucor Rouxii and other Species of Amylomycetes . . . 63
§ 241. Ragi and Tapej . . . 67
§ 242. The so-called Amylomyces Process 69

Section XII.—Form, Structure, and Chemical Composition of the Yeast Cell.

CHAPTER XLVI.—MORPHOLOGY AND LIFE HISTORY OF THE YEASTS.

§ 243. Position of the Saccharomycetes in the Botancial System . 72
§ 244. The Question of the Origin of the Saccharomycetes . . 76
§ 245. Bottom Yeasts 82
§ 246. Film Formation . . . 87
§ 247. The Ascospores 94
§ 248. Germination of Saccharomycetes Spores. Their Resistance to Heat. Pasteurisation . 99

CHAPTER XLVII.—ANATOMY OF THE YEAST CELL.

§ 249. The Cell Membrane . . . 106
§ 250. The Cell Nucleus . . . 109
§ 251. The Granules 112

CHAPTER XLVIII.—CHEMISTRY OF THE YEAST CELL.

§ 252 Chemistry of the Yeast Cell Nucleus 117
§ 253. Glycogen and Fat . . . 123
§ 254. Mucinous Substances of the Carbohydrate Group — The Gelatinous Network . . 127
§ 255. Albuminoid Mucinous Substances—"Head" and Frothy Fermentation 132
§ 256. "Break" and Clarification in Beer and Wine . . . 135

Section XIII.—Yeast Nutrition and Yeast Culture.

CHAPTER XLIX.—MINERAL FOODSTUFFS.

§ 257. Ash Content and Ash Analysis . 139
§ 258. The Importance of Lime . . 142
§ 259. The Phosphoric Acid Requirements of Yeast . . . 143
§ 260. The Importance of Sulphur . 146

CHAPTER L.—ORGANIC FOODSTUFFS. THE REQUIREMENTS IN RESPECT OF OXYGEN.

§ 261. Sources of Carbon . . . 148
§ 262. Inorganic Sources of Nitrogen . 151
§ 263. Organic Sources of Nitrogen . 154

CHAPTER LI.—CULTIVATION AND REPRODUCTION OF YEAST.

§ 264. Hansen's Method of Single-cell Culture 159
§ 265. Conditions of Cell Reproduction 164
§ 266. Consumption of Oxygen for Cell Reproduction and Respiration 169

Chapter LII.—The Effect of Certain Technically Important Chemical Influences on Yeast.

PAGE

§ 267. Copper and its Salts . . . 173
§ 268. Behaviour of Yeast Cells toward Alcohol 175
§ 269. Inorganic Acids and Salts . 178
§ 270. Organic Stimulants and Poisons 180

Section XIV.—Life History and Variability of the Saccharomycetes. Classification of the Saccharomycetes and Schizosaccharomycetes.

By ALBERT KLÖCKER.

Chapter LIII.—The Life History of Saccharomycetes in Nature.

§ 271. Fundamental Researches on the Life History of Saccharomycetes 183
§ 272. Later Experience on the Life History of Saccharomycetes . 186

Chapter LIV.—Variability and Heredity in Saccharomycetes.

§ 273. Temporary Variations . . 189
§ 274. Hansen's Researches on Asporogenation. The Production of Constant Varieties by Transformation 191
§ 275. Hansen's Experiments with Top and Bottom Yeast . . . 194
§ 276. Practical Results of the Researches on Variation Occurrence in Brewing Practice . 196

Chapter LV.—Classification of the Families Saccharomycetaceæ and Schizosaccharomycetaceæ.

§ 277. Introduction. Division of the Family Saccharomycetaceæ . 199
§ 278. The Genus Saccharomyces, with the Genera Hansenia and Torulaspora 202
§ 279. The Genera Zygosaccharomyces, Saccharomycodes, and Saccharomycopsis . . . 209
§ 280. The Genera Pichia and Willia. The Doubtful Genera Monospora and Nematospora . . 211
§ 281. The Family Schizosaccharomycetaceæ 216

Section XV.—Morphology, Physiology and Classification of Certain Technically Important Higher Ascomycetes and Allied Forms.

Chapter LVI.—Morphology and Subdivision of the Family Aspergillaceæ.

By PROF. DR. CARL WEHMER.

§ 282. Systematic Position and Classification of the Aspergillaceæ . 218
§ 283. The Genus Aspergillus . . 221
§ 284. Aspergillus Species with Simple Sterigmata 227
§ 285. Aspergillus Species with Branched Sterigmata (Sectio Sterigmatocystis) 237
§ 286. The Genus Penicillium . . 242
§ 287. The Species of the Genus Penicillium 246
§ 288. The Genera Citromyces and Allescheria 256

Chapter LVII.—Chemical Activity of the Aspergillaceæ.

By PROF. DR. C. WEHMER.

§ 289. General Review 259
§ 290. Saccharification of Starch . 260
§ 291. Acid Fermentations . . . 261
§ 292. The Fission of Disaccharides and Trisaccharides, Glucosides, and Polysaccharides (Starch excepted) 267
§ 293. Formation of Alcohol . . 272
§ 294. The Degradation of Proteids and their Derivatives . . 273
§ 295. Colouring-matters, Poisons, Oxidations, &c. 275

Chapter LVIII.—Mycosphærella tulasnei and Sphærulina intermixta, otherwise Cladosporium herbarum and Dematium pullulans.

By PROF. DR. G. LINDAU.

§ 296. Cladosporium herbarum . . 278
§ 297. Dematium pullulans . . . 281

Section XVI.—General Morphology, Physiology, and Classification of Technically Important Budding Fungi of the Group "Fungi Imperfecti."

Chapter LIX.—Torulaceæ, Pink Yeasts and Black Yeasts.

By PROF. DR. H. WILL.

§ 298. Historical, Delimitation, Derivation 286
§ 299. Occurrence, Dissemination, and Morphology of the Torulaceæ 290
§ 300. Physiology and Chemistry of the Torulaceæ 293
§ 301. Red Yeasts and Black Yeasts . 299

Chapter LX.—Mycoderma.

By PROF. DR. RICHARD MEISSNER.

§ 302. Species of Mycoderma . . 303
§ 303. Form, Dimensions, and Contents of Mycoderma Cells . . 304
§ 304. Reproduction of Mycoderma in and upon Various Nutrient Media 306
§ 305. Superficial Vegetations and their Attendant Phenomena . . 307
§ 306. The Destruction and Production of Acid in Nutrient Liquids by Mycoderma. 309
§ 307. Destruction and Formation of other Organic Substances by Mycoderma 311
§ 308. Influence of other Factors on the Vitality of Mycoderma . . 312

Chapter LXI.—Saccharomyces Apiculatus.

By PROF. DR. H. MÜLLER-THURGAU.

§ 309. History, Distribution, and Morphology 314
§ 310. Racial Differences . . . 317
§ 311. Conditions of Growth and Nutrition 318
§ 312. Fermentation Phenomena of Apiculatus Yeast . . . 320
§ 313. The Importance of Saccharomyces Apiculatus in Wine-making 324

Chapter LXII.—The Monilliæ and Oidia.

By DR. H. WICHMANN.

§ 314. Monilia, Sachsia, and Chalara . 330
§ 315. Oidium lactis and Allied Species 336

Section XVII.—The Enzymes and Enzyme Actions of Yeast.

Chapter LXIII.—Alcoholase.

By DR. RUDOLF RAPP.

	PAGE
§ 316. Historical Introduction	340
§ 317. Preparation of Expressed Yeast Juice	342
§ 318. General Properties of Expressed Yeast Juice	344
§ 319. Changes set up in Expressed Yeast Juice by External Physical or Chemical Influences or by Living Organisms	347
§ 320. Buchner's Zymase or Alcoholase	352
§ 321. The Position of Alcoholase with Relation to the other Enzymes	356

Chapter LXIV.—The Chemistry of Alcoholic Fermentation.

By DR. ARMINIUS BAU.

§ 322. The Chemistry and Chief Products of Alcoholic Fermentation	359
§ 323. The Non-volatile By-products of Alcoholic Fermentation, Glycerine, Isobutyleneglycol, Succinic Acid, Oxalic Acid, Lactic Acid	365
§ 324. Volatile Acids and Aldehydes as By-products of Alcoholic Fermentation. Influence of Oxygen on Fermentation	370
§ 325. Alcohols and Esters (Bouquet Principles) as Volatile By-products of Alcoholic Fermentation. Other By-products	375
§ 326. Sugars Susceptible of Direct Fermentation	381

Chapter LXV.—Enzymes Decomposing Disaccharides and Polysaccharides.

By DR. A. BAU.

§ 327. Invertase	384
§ 328. Maltase	388
§ 329. Melibiase	391
§ 330. Lactase	394
§ 331. Trehalase	396
§ 332. Raffinase	397
§ 333. Fermentation of Dextrin by Yeast (Amylase)	399
§ 334. The Autofermentation of Yeast	404

Chapter LXVI.—Endotryptase and Philothion.

By DR. M. HAHN AND DR. LAFAR.

§ 335. Endotryptase	408
§ 336. Philothion	415

Bibliography 417

Index 519

TECHNICAL MYCOLOGY.

SECTION X.

RUDIMENTS OF THE GENERAL MORPHOLOGY AND PHYSIOLOGY OF THE EUMYCETES.

CHAPTER XXXIX.

GENERAL MORPHOLOGY.

§ 217.—Articulation of the Thallus.

Already in the first volume (§ 22) the algæ and the fungi were arranged in a single group, that of the **Thallophytes**, in contradistinction to all other plants, the latter being classed in the group of **Cormophytes**. The distinguishing characteristics of these two groups were stated to be the absence in the former, and the presence in the latter, of an articulation of the body of the individual organism into leaf and stem. At the same time, it was mentioned that the corporeal form assumed by the *Thallophytes*, and differing fundamentally from the **cormus** of the *Cormophytes*, has received the general name of **thallus**. That intermediate forms between these two types should exist, and that the thallus of the highest *Thallophytes* should approximate to the cormus of the lowest *Cormophytes*, is perfectly natural and in accordance with the general laws of phylogenetic evolution.

Although the fungi, the only class of *Thallophytes* with which we are now concerned, do not exhibit division into leaf and stem, their thallus is not entirely destitute of all articulation. True, in one of the two chief divisions of the fungus family, the **Schizomycetes**, the articulation of the thallus is practically undiscernible, the individual organisms taking the form of globular or oval cells, or straight or bent rods of variable length. Should any extensive development of the thallus occur, this may almost invariably be regarded as either a malformation preceding death—*e.g.*, the branching of bacteroids (§ 195) and *Bacterium aceti* (§ 211)—or as an assemblage of several individual organisms giving rise to a deceptive appearance of articulation, as in the case of *Cladothrix* (§ 197). Again, in many species of bacteria, the colonies known as zoogloœa seem to exhibit a more or less well-developed articulation; but these cannot be considered as a thallus, since they represent assemblages of many uniform cells and not separate individual organisms.

The case is, however, different in the second division of the fungus family (§ 23), namely, the **Eumycetes** or higher fungi. These differ from the *Schizo-*

mycetes in universally possessing the faculty of forming **true branchings**. This characteristic resides in the immediate and uninterrupted connection between the plasma of the branch (or the oldest cell of same) and that of the main stem from which the branch proceeds. The divergence of form and the luxuriance of this branching vary in the different orders of fungi, and, in general, increase the higher we get in the system and the nearer we approach the *Cormophytes*. In

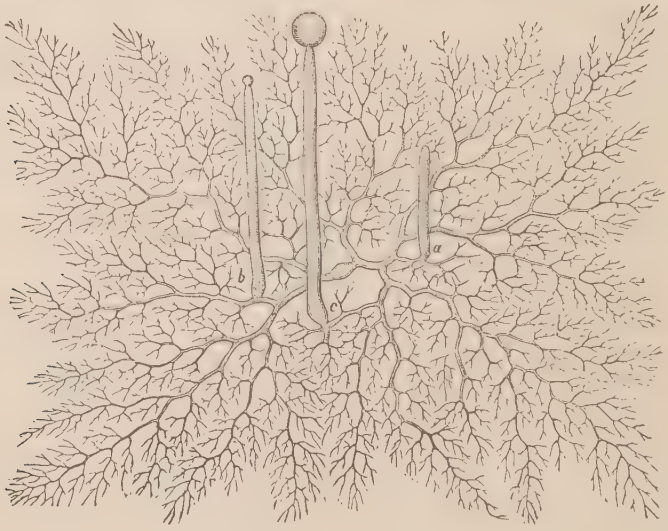


FIG. 91.—Thallus of Mucor mucedo.

Shows the unicellular mycelium sprung from the spore; together with three organs of fructification, *a*, *b*, *c*, in different stages of development, raising themselves from the mycelium. Magn. about 10. (*After Kny.*)

the lowest members, on the other hand, this tendency is often greatly simplified and restricted, thus approximating to the *Schizomycetes*.

A more careful examination of the thallus of the higher *Eumycetes*, even with the unassisted eye, will reveal something more than the existence of a more or less copious branching. It will soon be found possible to dissect the thallus into two parts (Fig. 91), which though intimately connected serve entirely different purposes: one, known as the **mycelium**, having charge of the nutrition and maintenance of the individual plant; whilst, on the other, or **organ of fructification**, devolves the task of reproduction, and therefore the maintenance of the species. This latter organ develops special cells or **spores**, which are mostly globular or oval, and from each of which, under favourable circumstances, a new individual of the same species can be produced.

The *mycelium* may therefore be defined as the portion of the thallus spreading in or upon the nutrient medium and extracting nutriment therefrom. It proceeds from a spore. As soon as this latter comes under the influence of circumstances favourable to its germination, it absorbs water and other nutrient materials from the surroundings, swells up more or less, and usually puts forth one or more **tubular buds** (Fig. 92). These continue to develop in two directions, increasing in length, and forming lateral branches which in turn continue to act in like manner. The name **hypha** is given to each of these branchings, and the whole group of *hyphæ* that have resulted from a single spore and serve to nourish the individual plant in question, is called the *mycelium*. The spore

may also germinate by the process of gemmation described in § 219. In some fungi the spores can only germinate in the one manner, whilst in others the germination is restricted to the other type. The mycelium, in order to fulfil its task, must continue to penetrate towards more remote portions of the nutrient medium; and accordingly, the hyphæ must progressively increase in length. Now this growth is confined to the apex of the hyphæ, *i.e.*, the part farthest from the centre of development. On the other hand, the parts nearest that centre quickly cease to extend and branch; consequently, the *Eumycetes* exhibit acrogenous growth. This behaviour constitutes another feature of difference between *Eumycetes* and *Schizomycetes*, since, in the latter, the growth of the cells is not restricted to one end only, but proceeds by the extension of the whole body.

FIG. 92.—Mucor mucedo. Germinating spore, which has already put forth two buds. Magn. 300. (*After Brefeld.*)

Examination of the mycelia of a large number of species of *Eumycetes*, under a power of about 100 diameters, soon leads to a separation of these specimens into two groups: the one comprising species whose mycelium, however large and extensively branched, consists of only one single cell; whilst the other group contains the species wherein the mycelial hyphæ are subdivided into cylindrical parts of variable length by transverse walls (**septa**) perpendicular to the longitudinal axis. This fundamental and highly important difference constitutes the chief basis for the separation of the *Eumycetes* into two main subdivisions; *Eumycetes* with a unicellular mycelium on the one hand, and *Eumycetes* with a septated mycelium on the other. In mycelial structure and several other particulars, the members of the first subdivision bear a remarkable resemblance to certain unicellular algæ, for which reason they have received the name **Phycomycetes**, or *algic fungi*. On the other hand, the *Eumycetes* of the second group, with septated mycelia, bear the group-name **Mycomycetes**. In comparison with the others these latter stand on a higher plane of development, and are almost exclusively aerial; whereas the majority of the *Phycomycetes* still incline to subaqueous existence. The following scheme will easily fix the foregoing classification in the reader's memory:

- Vegetable Kingdom
  - *Cormophyta*
  - *Thallophyta*
    - *Algæ* (producing chlorophyll)
    - *Fungi* (*Mycetes*) (not producing chlorophyll)
      - *Eumycetes* (Acrogenous true branchings)
        - *Mycomycetes* (septated mycelium)
        - *Phycomycetes* (unicellular mycelium)
      - *Schizomycetes* (Fission fungi)

§ 218.—The Typical Mycelium.

We will now consider the **development of the mycelium of a Mycomyces from its spore**, Fig. 93 helping to make this clear. Soon after the tubular buds have sprouted from the spore, a septum forms between the spore and each of the buds. The tube then increases in length, and develops in its interior a septum which divides it into two cells, the one nearer the spore (or centre of growth) being termed the **inner cell**, whilst the outer one is called the **terminal** or **crown cell**. Now, whereas the inner cell ceases to increase in length the crown cell continues to grow longitudinally, and in turn develops a septum, whereby an inner cell (of the second order) is again formed; and this operation is repeated at convenience. Meanwhile the inner cells are not inactive, since, although they do not increase in length, they throw out lateral projections, which develop into branches separated from the inner cell by septa. These branches grow

longitudinally, and separate into a crown cell and a secondary inner cell by developing another septum, an operation repeated by the crown cells as often as external circumstances will admit. This faculty of the inner cells of the first order is also shared by those subsequently formed, so that we have lateral branches of the third, fourth, and other orders. The whole of these hyphæ or mycelial threads together constitute the mycelium. The serial order in which

FIG. 93.—Mycelial development of a Mycomyces (the ordinary bread mould, *Penicillium glaucum*).

A, the ripe spore. *B* and *C*, the same, with respectively one and three tubular off-shoots. In *D* each of these has become separated from the spore by a septum, *s*. In *E* each tube has become divided by the formation of a second septum, *s'*, into a terminal cell (*e*) and an inner cell (*b*), whilst branching has commenced in two places. *F* shows each of the three tubes developed into a main branch (I. to III.), which in turn has thrown out lateral branches of the first to the third order (1, 2, 3). Magn. 400. (*After Zopf.*)

the inner cells begin to throw off lateral branches is, as a rule, in accordance with their age, the oldest starting first. Consequently, the development of the mycelium proceeds laterally from the spore (the "basis") towards the periphery or apex of the mycelial thread, such a mode of growth being termed **basifugal** or **acropetalous**. Again, the lateral position of the branches in question is, as a rule, very uniform; those from the inner cells of odd-numbered orders all branching from one (*e.g.*, the left) side, and all those springing from inner cells of even-numbered orders appearing on the opposite (*e.g.*, right) side of the respective inner cells. When the branching from any given cell is confined to a single lateral off-shoot the system is termed **monopodial**, the main and branch cell together being called a **monopodium**.

The progress of mycelial development in a *Phycomyces* naturally differs from

that just described, inasmuch as no septa are formed under normal circumstances; consequently there is, in this case, no separation of the elongating tubular bud into inner and crown cells. In such event the resulting mycelium consists, as already stated, of a single, many-branched, tubular, non-septated cell, such as is shown in Fig. 91.

The foregoing statement that the inner cells do not play any further part in the mycelial growth of the *Mycomycetes*, inasmuch as they neither extend in length nor develop septa, may be taken as the rule. There are, however, exceptions, septation, accompanied by elongation, frequently occurring within the inner cells in the event of abnormal conditions of nutrition. This phenomenon is termed **intercalary growth**, or **intercalary septation**, to distinguish it from **acrogenous growth.**

If, in the absence of external causes of hindrance, the growth of the mycelium is able to proceed equally in all directions, a stellar system of radial, branched threads, with the spore as a centre, is the result. This form of growth was termed a **typical mycelium** by Zopf. The practical worker in a mycological laboratory can obtain such typical mycelia in a youthful condition, and conse-

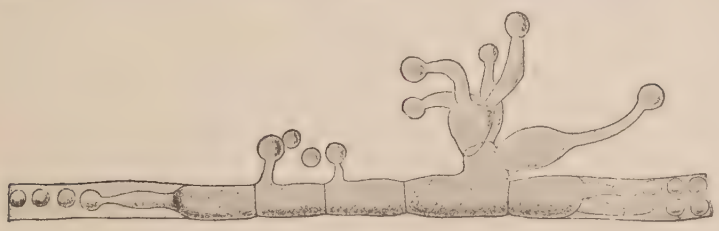


FIG. 94.—Botrytis cinerea.

Intergrowth. Each of the two penultimate cells of the depicted fragment of mycelium has grown into its neighbour, and there become separated into globular cells. The central cell of the mycelial thread has put forth abnormally developed organs of fructification. (*After P. Lindner.*)

quently easy to survey, if he re-examines, after a lapse of one or two days, the plate cultures (§ 85) that have already been examined for the purposes of mycological analysis (*e.g.*, of water, milk, beer). During the first investigation the spores of all kinds of mould fungi from the air will have settled on the solid nutrient medium, each of them then germinating to form a mycelium, and thus yielding, as it were, a self-prepared culture.

Mention must here be made of one of the various instances of irregular mycelial development, since it will have to be referred to on a subsequent occasion: this is the phenomenon of intergrowth. It is caused by one of the cells in a mycelium putting forth a branch into the interior of an adjoining cell, so as to displace the intervening septum. The invader may then become divided into cells within the plasma of the invested cell, with the result that an inexpert observer may easily be led to believe that endogenous spores are present. An example of this growth is represented in Fig. 94. Another will be found in a later section dealing with *Dematium pullulans*, and a third in the case of *Oidium Ludwigii*, Hansen, occurring in mucilaginous discharges from trees (§ 248), and investigated by W. HOLTZ (I.). This was probably also the method of formation of the alleged spores observed by EDM. KAYSER (V.) within the hyphæ of an unknown mycelial fungus isolated by him from fermenting pine-apple juice. Intergrowths also occur in the sporangia of several fungi.

In the case of a large number of fungi, the development of the mycelium ceases with the formation of the branched hyphæ, the ensuing process being the elaboration of organs of fructification. Fungi exhibiting this class of simple

mycelial structure are classed under the generic name **Hyphomycetes** or **Thread Fungi.** The term **Mucedinæ**, occurring in the French and English literature, expresses about the same thing. It may be remarked in passing that several botanists, *e.g.*, Strasburger, Noll, Schenck, and Schimper, in their botanical text-book, employ the name *Hyphomycetes* in a far wider sense, namely, to include the whole of the *Eumycetes*, the reason for this being that the production of hyphæ is characteristic of these fungi, and constitutes a fundamental distinction between them and the other divisions of the fungus family, the *Schizomycetes* in particular. Nevertheless, in the following pages we will apply the term in its more restricted sense.

In many of the other classes of *Eumycetes*, the development of the mycelium does not cease at the stage we have described as the typical mycelium, but extends further, to the production of *aggregations*, of the forms known as **mycelial threads** and **mycelial films.** A combination of these two forms constitutes the large bodies known in colloquial language as "mushrooms" or "fungus"; the botanist, however, terming them **fungoid bodies.** The capacity of forming such bodies, upon or within which the organs of fructification are situated, is confined to the most highly developed species of fungi. An example is given in Fig. 95. This, however, is only one variety (though appearing in numerous modifications) of the coalescence and intertwined growth of hyphæ, another form being that of the so-called **sclerotium** or **hard mycelium.** The well-known ergot of rye, which will be more closely described in the last section but one, forms an example of a sclerotium. This is constructed of closely intertwined hyphæ, furnished with a store of nutrient material, and constituting a hard permanent form, which, after a variable period of quiescence, awakens to active life, puts forth organs of fructification, and is then able to await and utilise the occurrence of favourable conditions, in order to effect the reproduction of the individual from which it has originated. An observation on the artificial production of such permanent forms has been communicated by J. Ray (I.). Among the foodstuffs accumulated in the cells of the sclerotium, special importance attaches to glycogen (§ 253) as the source of easily liberated chemical energy and abundant disengagement of heat. This substance was first observed—without, however, being specially named—by A. de Bary (I.) in the sclerotium of *Coprinus stercorarius*; and it was afterwards found, by W. Rothert (I.), in that of *Sclerotium hydrophilum*. A thin section of sclerotium, or of a fungoid body—both of which are, as already stated, composed of a network of hyphæ—exhibits under the microscope an appearance similar to that of the parenchyma of higher plants, *e.g.*, a section through the flesh of an apple. On account of this similarity these networks of hyphæ have received the name *pseudoparenchyma*, which, however, is not intended to express any further likeness, whether in respect of the mode of formation or physiological purpose. It is perhaps superfluous to emphasise that the mycelia of this class of fungi consist merely of hyphæ when in the earliest stages of existence, and consequently

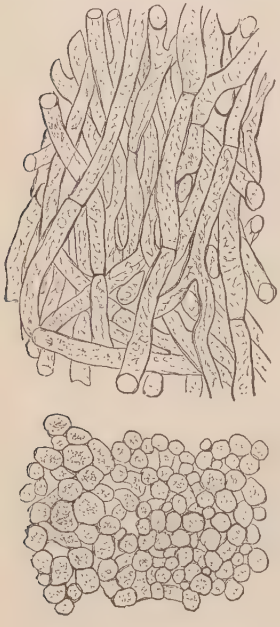


Fig. 95.—Boletus edulis.

Longitudinal section (above) and transverse section (below) through the fruit stem ("fungus") of Boletus edulis. Magn. 300. (*After Strasburger.*)

at such times are indistinguishable in this respect from the mycelia of the *Hyphomycetes*.

§ 219.—The Gemmating Mycelium.

The application of the name "typical" to a mycelium growing in the manner described in the preceding paragraph indicates the possibility of other methods of growth, manifesting themselves as modifications and simplifications of this form. Of these the most important, from our point of view, is the **gemmating mycelium**, the development of which proceeds in the following manner (Fig. 96): The germ-cell, or mother-cell, puts forth a protrusion which, however, instead of enlarging to a tube as in the case of the incipient typical mycelium, assumes a form resembling that of the parent-cell and therefore termed a bud. The daughter-cell then becomes divided from the parent by a septum, which subsequently splits into two layers and enables the two cells to separate. In many instances the parent-cell puts forth only a single bud, but in others two or more. As soon as the daughter-cell has attained the size of the parent, it is then able to behave in turn like the latter, and itself put forth a bud (of the second order), from which again proceeds another bud (of the third order), and so on.

If the parent-cell—as is the case, for example, in most kinds of yeast—be globular, oval, or lemon-shaped, the daughter-cell will also usually be of similar form, and is then termed a short bud. Such globular buds are referred to in the older literature (and occasionally even now) as **spherical yeast**, more particularly in the case of *Mucor*. If, on the other hand, the parent-cell be of elongated form, the daughter-cells issuing therefrom will preferentially develop in a longitudinal direction from the start, and thus form elongated buds. Most of the species of *Mycoderma* afford examples of this type. Fungi with gemmating mycelia of this kind are therefore, in this respect, intermediate to the fungi with typical mycelia.

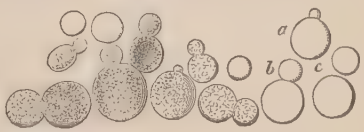


FIG. 96.—Gemmation of a Torula in beer wort.

At (*a*) one of the cells has just put forth a tiny bud. At the end of 1½ hours (*b*) this has become considerably larger. After another two hours it has grown to half the size of the parent cell and has already separated from the latter. Magn. 1000. (*After Hansen.*)

The above-mentioned double stratification of the septa between the cells produced in the foregoing manner permits these cells to enjoy an independent existence, and consequently enables them to be separated from one another. In many instances this actually occurs, and consequently the nutrient medium wherein this takes place will exhibit a comparatively large number of single cells. Conversely, in other instances, the successively developed buds remain connected together, forming a cellular aggregation (Fig. 97). In the older literature, such aggregations, when composed of globular cells, and therefore resembling a series of small knots (Lat. *Torula*), were generally named **Torulæ**. This was afterwards employed as the generic name for a number of species, some of which are capable of exciting alcoholic fermentation and will be described in a later section. An example of these is given in Fig. 98.

The form of the gemmæ from one and the same species is also dependent on the temperature and the conditions of nutrition, as has been shown by E. Ch. Hansen in the case of beer yeasts and wine yeasts. These, when submerged in beer wort, develop mycelia constructed of short gemmæ; whereas, when cultivated on the surface of the liquid, and therefore in presence of abundance of air, they form mycelia composed of elongated buds. Further particulars of this will be found in § 246.

The formation of mycelia composed of gemmæ was first observed in the case of yeast fungi, and was regarded as a method of development peculiar to these organisms. Ball (I.), however, in 1857, showed that this phenomenon also appears in certain species of *Mucor* (*see* chapter xliv.) when submerged in a nutrient solution containing sugar. For more precise observations of this phenomenon in the case of *Mucors* we are indebted to Brefeld (XVI.). With *Mucor racemosus*, the carbon dioxide collecting in the nutrient fluid acts upon the cells, by which it has been produced, in such a manner that the latter put forth none but spherical gemmæ, and therefore grow, not to a long and many-branched, unicellular mycelium, but to one composed of stumpy gemmæ. On the other

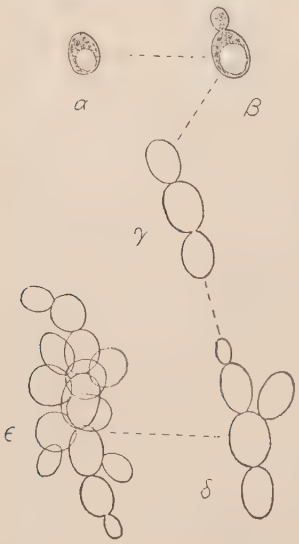


Fig. 97.—Saccharomyces pyriformis, Ward.

Cell α, embedded in a hanging drop of ginger-beer gelatin and kept at a temperature of 15° C., threw out a bud (β) within 4½ hours. At the end of another 14 hours three normal cells (γ) were present, which grew to the aggregation δ in another 10 hours. This in turn had developed into the colony ε in 13½ hours more. (*After M. Ward.*)

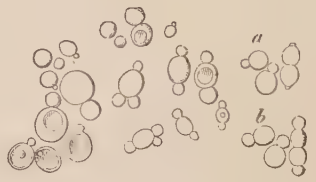


Fig. 98.—Torula.

Specimen grown in beer wort. *a* represents a group of gemmating cells, the condition of which, after the lapse of an hour, is shown at *b*. Magn. about 1000. (*After Hansen.*)

hand, *Mucor mucedo* treated in the same manner does not produce similar gemmæ, though, according to Brefeld (XVI.), its spores, when placed in a nutrient solution rich in citric acid, swell up to large globules from whence proceed a number of similarly formed daughter-cells, which, however, finally perish. Numerous other fungi are also credited with the same capacity.

Now, all the *Eumycetes* capable of forming mycelia of this description can be divided into three groups. To the **first group** belong all such as, under normal conditions of nourishment, develop exclusively in the form of mycelial aggregations of gemmæ. They are therefore known as **budding fungi**. This group comprises, without exception, all the *Saccharomycetes* concerned in the fermentation industries, together with the *Mycodermæ*, *Torulæ*, &c. The **second group** consists of such *Eumycetes* as, under normal conditions, are equally well able to develop either a filamentous mycelium, or one of gemmæ. These organisms also are occasionally termed budding fungi, examples of the class being afforded by certain species of *Monilia* (Fig. 99), *Dematium*, and others. Finally, the **third group** includes all the fungi which usually produce a filamentous mycelium, the

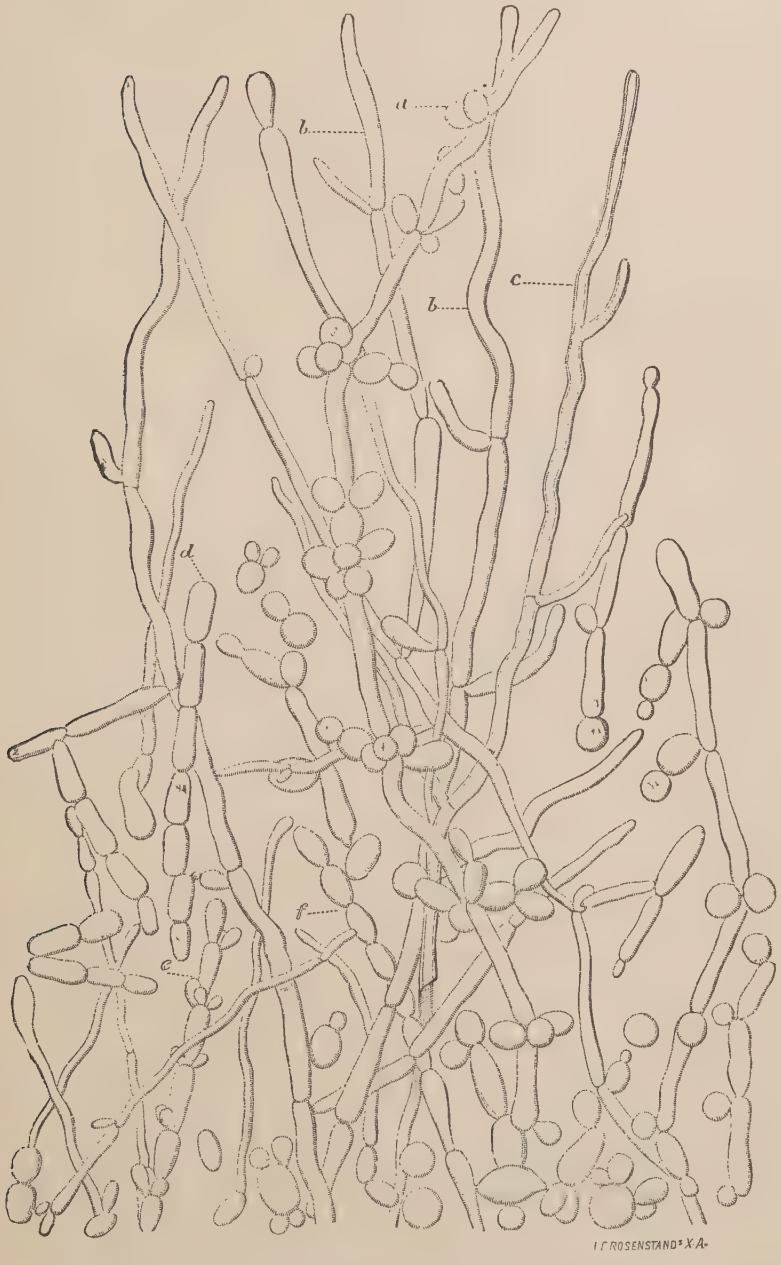


FIG. 99.—Monilia candida

Film vegetation on beer wort. Typical mycelia are present at *b* and *c*; the bud form at *a*, *e*, and *f*. Magn. about 1000. (*After E. Ch. Hansen.*)

other form being only developed under abnormal conditions of nourishment. To this class belong certain species of *Mucor*, such as *Mucor racemosus*, *M. tenuis*, *M. erectus*, *M. fragilis*, *M. spinosus*, *M. ambiguus*, *M. circinelloides*, *M. alternans*, and numerous others.

However, the term "budding fungus," applied to any fungus on account of the above-mentioned behaviour, does not, in general, convey any definite information with regard to the position of that organism in the botanical system. As a matter of fact the species included in the group of budding fungi belong to very different families of the fungus kingdom.

With regard to the **anatomy and chemistry** of the *Eumycetes* cells a few general observations may be made in this place. Naked cells, *i.e.*, cells destitute of membrane, occur (as motile spores) in only two—for us unimportant—subgroups of Hyphomycetes, viz., the *Chytridiaceæ* and *Oomycetes* mentioned in subsequent paragraphs. Full information in connection with their chemical character will be found in Chapter xl. As in other cases, so also with the *Eumycetes*, the cell contents (plasma) can be divided into the nucleus and the cytoplasma with its inclusions. From the general study of cells, as treated in all botanical hand-books, and more particularly in A. Zimmermann's monograph (III.), the reader will be aware that every normal living vegetable cell contains at least one nucleus. This was first discovered by Robert Brown (I.), who, in 1831, found nuclei in the cells of higher plants, whereas their presence in the cells of fungi was only ascertained by Fr. Schmitz in 1879 (I.). It was subsequently found that the mycelium of *Hyphomycetes* is, without exception, rich in nuclei. On the other hand the mycelial cells of *Mycomycetes* contain either only a single nucleus (as in the *Saccharomycetes*), or several, as is the case, *e.g.* (according to Fr. Schmitz (II.)), with the mycelial cells of *Penicillium glaucum*, and those of the sclerotial medulla of *Claviceps purpurea*, which contain one, two, or more nuclei; and the mycelial cells of *Peziza convexula* contain several nuclei throughout. The active part played by the cell nuclei during the development of the mycelium, sporulation, and germination, has been more closely investigated by G. von Istvanffi (I.). The reproduction of the nucleus by fission (§ 46) is mostly direct among the *Eumycetes* (*e.g.*, *Penicillium*). Nevertheless, cases of **karyokinesis** have been observed, first by Sadebeck (II.) and afterwards by Fisch (I.). Istvanffi was unable to discern any fusion of the nucleus, as recorded by Strasburger, Zopf, and Fisch, in the formation of the oogonia of the *Oomycetes* (see § 220). It may be mentioned that the same observer also ascertained that the size of the cell nuclei in one and the same species varies in accordance with the importance attained by the corresponding parts of the thallus. Fuller particulars respecting the anatomy of nuclei, especially those of yeast cells, will be found in § 250, and on the chemistry of the subject in § 252, these details supplementing in more than one point those already given. No investigation seems to have, as yet, been conducted with regard to the presence of **centrosomes** in fungi, *i.e.*, those organised constituents of cell plasma which, of late years, have become so important in the cellular physiology of animals and the higher plants.

Observations of a general character in connection with the anatomical structure of cytoplasm, and the chemical constituents of which it is composed, will also be frequently encountered in the following chapters.

Many of the Eumycetes exhibit **brilliant colouring**. Full particulars regarding the colouring-matters concerned will be found in Zopf's (X.) handbook; and these may be supplemented by the following reference to a technical utilisation of one of these fungous pigments. To impart a red colour to rice wine, to various spirituous liquors, bread, cakes, and to the fish held in such high esteem (under the name of Macassar or red fish) in the Malay Archipelago, the Chinese

employ a colouring-matter extracted from a red *Hyphomyces*, which they cultivate on boiled rice. The fungus grows with vigour on this medium, and imparts thereto a red coloration ; and the dried cultures, to which a preservative addition of arsenic and mustard oil is made at the time of preparation, form an article of commerce under the name Ang-Khak. C. Went has named this fungus *Monascus purpureus*, and identified it as a member of the *Thelebolea* family of *Ascomycetes* (§ 220). The red colouring-matter was first examined by H. C. Prinsen Geerligs (IV.); and W. G. Boorsma (I.) afterwards found that two red dye-stuffs, α-Oryzæ-Rubin and β-Oryzæ-Rubin, can be extracted from Ang-Khak, in which they are jointly present to the extent of about 1.6 per cent.

§ 220.—Fructification by Sporangia.

The separation of the thallus into two portions: the mycelium on the one hand, and the organ of fructification on the other, can be readily found in all *Eumycetes* of anything like a high stage of development; though in the lower members of the system they are only very indefinite ; and may even be entirely absent, in which case the mycelium itself is converted into an organ of fructification.

The chief function of the organs of fructification is the production of structures capable of developing into new individuals of the same species as the parent organism. In the case of fungi these structures, which consequently serve for **direct propagation**, are termed **spores** ; and, in accordance with the method of their production, are divided into four groups :

(1) *Endospores*, or *Gonidia*.
(2) *Zygospores*.
(3) *Exospores*, or *Conidia*.
(4) *Chlamydospores*, or *Gemmæ*.

Setting aside, for the present, the fourth group, which occupies a position apart, it may at once be stated that, in all the more highly developed *Eumycetes*, spores of the other three groups are produced by a special organ, which branches off from the mycelium as a fruit-bearing stem : the **aerial hypha**. According to the species of the fungus and the external conditions, each of these stems may produce either one or more spores.

To take first the case of the **Endospores**: these, as the name implies, are formed within the fruit-bearing organ, or a special part thereof. Except in the very lowest species of endosporogenic *Eumycetes*, in which the mycelium itself is converted into an organ of fructification, and its contents into spores, the process takes its course along the following main lines: an upright aerial hypha (*see* Fig. 91) branches off from the mycelium, and, as it proceeds to attain full growth, enlarges at the upper extremity to a usually spherical or bottle-shaped bubble : the *sporangium*, so called because in it the spores are formed. Before this happens, however, a septum is developed between the sporangium and the aerial hypha, which wall, in many cases, is not straight but curved, and projects some distance within the sporangium, as though actually an enlargement of the tip of the hypha. In some instances it assumes the form of a short pillar (Fig. 100), on which account it has received the general name *columella*. The endospores are then formed, as free cells, out of the contents of the sporangium, either the whole of these contents being drawn upon for this purpose, or only a portion as in the case of the Mucors (§ 236). Fig. 100 represents a ripe sporangium filled with spores.

In one of the three classes of *Phycomycetes*, namely the *Oomycetes*, two kinds

of sporangia, differing in structure and behaviour, are produced. The members of the one kind are termed **zoosporangia**, or **motile sporangia**, their (naked) ciliated spores issuing forth on the rupture of the sporangium, whereupon they are known as zoospores. In the other kind of sporangia there are formed—as the result of sexual fructification—large, tough-walled, quiescent spores to which the name **oospores** is given. The capacity of producing sporangia of this latter kind, which are known as **oogonia**, belongs exclusively to the *Oomycetes*, and is their distinguishing character; whereas, on the other hand, zoosporangia and zoospores are found even in the second (lowest) class of *Phycomycetes*, viz., the *Chytridiaceæ*. Zoospores occur only in these two classes, the genera of which are sub-aqueous in habit, either exclusively or during a certain period of their existence. On the other hand, the third class of *Phycomycetes*—the *Zygomycetes*—being preferably adapted to an aerial existence, is without these consequently unnecessary organs. Still less are the latter required by the more highly developed *Mycomycetes*.

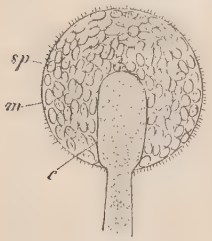


FIG. 100.—Mucor mucedo.

Sporangium in optical longitudinal section. Here *m* is the sporangial membrane; *c*, the columella; *sp*, the endospores. Magn. 225. (*After Brefeld.*)

It will be found of great utility to examine how far **sporangial fructification** extends throughout the *Eumycetes*. In the members of the lower sub-kingdom, the *Phycomycetes*, the faculty of producing endogenous spores is universal. On the other hand, this faculty is present in only a single class of *Mycomycetes*, namely, the *Ascomycetes*, or tube fungi, so called because the sporangium assumes a particular form, to which the name ascus (or tube) has been given. The ascus differs from the sporangium, of which it is a higher development, both in exhibiting a more definite form, and also in the number, shape, and method of formation of the contained spores (ascospores). Fuller particulars on this point will be given in § 243; since the majority of yeasts are Ascomycetes, and therefore require more thorough consideration.

The information already given renders it possible to enlarge, as follows, the scheme laid down in § 217 for the subdivision of the Eumycetes:

				Class.
Eumycetes (Acrogenous fungi)	*Mycomycetes* (with septate mycelium)	*without* endogenous spores		All other *Mycomycetes*
		with endogenous spores		*Ascomycetes*
	Phycomycetes (aseptate mycelium)	*without* oogonia		*Zygomycetes*
		without zoosporangia, but producing zygospores		
		with oogonia and zoosporangia		*Oomycetes*
		with zoosporangia		*Chytridiaceæ*

The cause of the upward growth of the aerial hyphæ—both those bearing sporangia, and those putting forth the conidia to be described later on—has already been the subject of repeated investigation. What is it that causes these hyphæ to raise themselves from the mycelium and extend vertically upwards? In accordance with its destiny, the aerial hypha draws on the rich store of material in the mycelium, for the purpose of producing spores and filling them with an accumulation of matter. This material, being taken up in the dissolved state, needs to be freed from solvents in order to secure its deposition; and the process can only be carried out on a large scale in an environment comparatively poor in moisture, a condition that is only fulfilled *above*, but not *within*, the aqueous or damp nutrient medium. Consequently the fruit-bearing aerial hyphæ

grow upwards into the air. This turning away from water is termed **negative hydrotropism**, and was first recognised, by J. Wortmann (XIV.) in 1881, in the aerial hyphæ of *Phycomyces nitens*. Communications on the same point having also been published by L. Errera (IX.), the above-mentioned state of things was afterwards recognised by G. Klebs (I.) on the basis of his researches on the conidiferous hyphæ of *Aspergillus* (*Eurotium*) *repens* and the sporangiferous hyphæ of *Sporodinia grandis*. That other functions, such as heliotropism (*see* § 233), may be manifested concurrently with negative hydrotropism is a matter of course. In connection with the dependence of sporangiation upon the conditions of nutrition, thoroughgoing investigations were conducted by Klebs (I.) on *Rhizopus* (*Mucor*) *stolonifer*; and this worker ascertained the definitive factor to be the percentage of moisture in the superincumbent atmosphere. J. Bachmann (II.) then showed that *Mortierella van Tieghemii* (*see* § 237) produces spores only when sown on a solid (not liquid) nutrient substratum, and then only provided the temperature does not fall below 20° C.

§ 221.—Fructification by Zygospores.

Zygospores are the result of the encounter of two hyphæ, or the fusion of two cells, and are produced in the following manner (Fig. 101). Two of the mycelial hyphæ coalesce, the crowns simultaneously swelling up in the shape of a club containing a considerable accumulation of plasma. As soon as the ends of the two hyphæ come into contact (1) they flatten and an intergrowth of the membranes occurs at this spot, whereupon a septum is developed in each of the clubbed ends, which are thereby separated from their respective hyphæ. Each is thus divided into two parts: a terminal cell, to which the name **copulation cell** or **gamete** is given (2 *a*), and an interior cell, or **suspensor** (2 *b*) supporting the gamete. The partition wall between the two terminal cells is then absorbed, and the contents of these latter fuse to form a new, uniform structure: the **zygospore** or **zygota** (3 *a*). At first the outside still continues to present the appearance of two cells, but the contour soon becomes rounded off. The membrane thickens and exhibits a separation into an inner (endosporium) and an outer case (exosporium), the latter turning very dark in colour and becoming lumpy or warty on the outside. The cell contents surrounded by the endosporium contain large quantities of accumulated nutrient material.

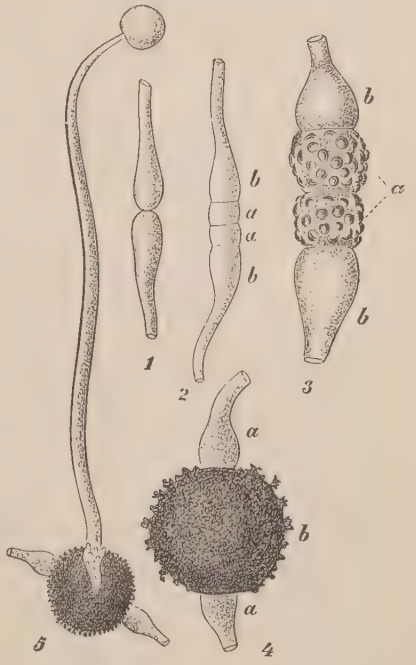


Fig. 101.—Mucor mucedo.

Formation of the Zygospores.

1. Two hyphæ in terminal contact.
2. Articulation into gamete *a* and suspensor *b*.
3. Fusion of the gametes *a*; the membrane thickens.
4. Ripe zygospore *b* supported by the suspensors *a a*. (Magn. of 1–4, 225.)
5. Germination of the zygospore to a sporangium stem. Magn. about 60. (*After Brefeld.*)

Sooner or later the zygospore becomes detached from the suspensors, and then leads a separate existence. Being a resting cell it can, if necessary, remain

quiescent for a considerable time, and then germinate on the recurrence of favourable external conditions.

The production of zygospores in the manner just described has been regarded by some mycologists as a sexual process; whilst other workers contest the admissibility of such an hypothesis. Whichever is right, one thing is certain, namely, that a structure very similar to a zygospore can be produced without cell fusion of the kind described. In such event the resulting body is called, for the sake of distinction, an **azygospore** or **parthenospore**. This kind of spore can be formed in several ways, one of them differing from the foregoing merely in that the partition between the gametes is not absorbed, and consequently no fusion of the cell contents takes place, each gamete ripening apart to form a spore; *i.e.*, two azygospores are produced. An example of this type is given in Fig. 102 (*a*). In other instances, however, the process is further simplified, inasmuch as no contact occurs between the two club-ended hyphæ, but each (or one) of the gametes (*b*, Fig. 102) develops of itself into an azygospore. In still

a *b*

Fig. 102.—Formation of Azygospores in Mucor erectus. (*After Bainier.*)

another instance, even the endeavour of two hyphæ to approach one another is absent, the azygospores forming quite alone and independently at the extremities of branches; an example of this method of sporulation is afforded by *Mucor tenuis* (§ 235).

Ehrenberg, in 1829, was the first to observe zygospores, the organism examined being *Sporodinia grandis* (§ 235), wherein these spores attain a diameter of about 0.25 mm. It was not, however, until 1864 that the method of production and germination was made clear by the researches of A. de Bary. Seven years later, Brefeld (I.) established the new order (class) of the *Zygomycetes*, comprising all the fungi capable of producing zygospores. These are all, without exception, *Phycomycetes*. In addition to this faculty, which distinguishes and separates them from all other fungi, the different genera of the *Zygomycetes* have, in common, various other properties which reveal their mutual relationship and therefore justify their classification into a special group, as expressed in the scheme drawn up a few pages back. This class of the *Zygomycetes* is the only one among the *Phycomycetes* that possesses any importance for the fermentation industries; it will be found treated in section xi.

The conditions under which the formation of zygospores occurs must not be left out of consideration, since they show that, here as in other cases, the development of fungi, like that of other living organisms, is the result of two factors; inherited properties and the sum of external active forces. The statement that zygospores are encountered solely in the class of the Zygomycetes does not imply that these fungi rely exclusively on the organs in question for their reproduction. On the contrary, this method of reproduction is rare, the ordinary and most frequent method being by sporangial or conidial fructification, as will be more specifically shown in chapter xliii. At present we will consider the influence of external conditions on the occurrence of one or the other method of fructification. In the case of *Sporodinia grandis*, G. Klebs (II.) has shown the principal factor to be the proportion of moisture in the surrounding

air. When this approaches the limit of saturation, zygospores alone are produced; but on the moisture being diminished, sporangia are formed as well; and finally, the latter are exclusively produced when the relative moisture has fallen to about 65 per cent. so that active transpiration can occur. The chemical composition of the nutrient substratum is also of influence, more particularly in that, in presence of an excess of nitrogenous substances, sporangia alone, and no zygospores, are formed, the last-named requiring the presence of suitable carbohydrates for their production. It is therefore particularly interesting to observe how decisively this fungus discriminates for this purpose between individual isomeric substances. Thus zygospores are produced when the available carbohydrate consists of mannite or dulcite ($C_6H_{14}O_6$), dextrose, levulose, galactose ($C_6H_{12}O_6$), saccharose, maltose ($C_{12}H_{22}O_{11}$), or dextrin; whereas sporangia alone are produced when the nutrient substratum contains one of the following carbohydrates: sorbite ($C_6H_{14}O_6$), sorbinose ($C_6H_{12}O_6$), lactose ($C_{12}H_{22}O_{11}$), raffinose, isodulcite or erythrite. It has also been found that conditions adverse to the formation of zygospores favour that of azygospores.

According to observations communicated privately by E. Ch. Hansen, the production of zygospores in *Sporodinia grandis* (as well as in a hitherto undescribed species of *Mucor*) is not so strictly dependent on the fulfilment of such conditions, but occurs readily and without any special experimental preparation. On the other hand, it cannot, so far, be induced at all in the case of several other species of *Zygomycetes*.

§ 222.—Fructification by Conidia.

The nature of this fructification and of its fundamental characteristic of differentiation from sporangial fructification lies in the circumstance that the spores, instead of being enclosed in a cell, separate by constriction from the fruit-bearing stem (conidiophore) externally, and are therefore known as **exospores**. In so far as the behaviour of the conidiophore is concerned the process may go on according to two different types, which are diagrammatically represented in Figs. 103 and 104. Let us first consider type I. with reference to Fig. 103. The upper extremity of the conidiophore, *a*, which has sprung from the mycelium, puts forth (*b*) on its crown an enlargement, which then (*c*) becomes bounded by a septum, thus forming the first exospore. The conidiophore (*d*) then stretches a distance equal to the length of a spore, and (*e*) undergoes constriction so as to form a second spore. This operation may be repeated a second time (*f*, *g*), and even more often, thus forming a chain of spores (*h*), the topmost of which (1) is the oldest, and the lowermost (4) the youngest. The serial order of their production is arranged from the tip towards the base of the conidiophore, or the point where it branches from the mycelium; and consequently the conidia are said to be **basipetal**. The ultimate length of the conidiophore is the same as at the outset no matter how many spores have been formed thereon. The surmounting chain of conidia breaks away very easily; even a slight draught of air being able to bring this about, and thus

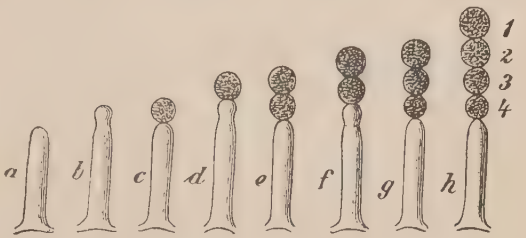


FIG. 103.—Diagrammatic sketch of the formation of conidia, according to type I.

For explanation *see* text. (*After Zopf.*)

distribute the spores in the form of a fine dust. Owing to their similarity to this latter the exospores have received a second appellation, and one by which they are more generally known, viz. **conidia** (from the Greek κονία = dust). An example of this type is afforded by the well-known bread mould, *Penicillium glaucum*.

In contrast to the foregoing process, wherein the conidiophore alternately increases in length and constricts to form a spore, is the second type, illustrated in Fig. 104. Here the conidiophore (*a*) ceases to grow in length as soon as the first spore (*b*) has been formed by constriction at the apex. This spore, when fully developed (*c*), puts forth a protrusion (*d*), which then grows to a second spore (*e*). This in turn continues the task of its predecessors, and consequently forms a third spore (*f*), which develops a fourth (*g*), and so on. Hence, in this type, the serial order of production of the spores (*h*) is the reverse of that in the first type, *i.e.*, proceeds from the base towards the apex, and is therefore **basifugal** or **acropetal**. In contrast to those of the first type these spores exhibit a capacity and tendency for throwing off spores laterally as well as from the apex, so that the operation, when repeated, gives rise to the formation of branched conidial clusters. An excellent example of this is furnished by *Cladosporium herbarium*, which will be described in a later chapter.

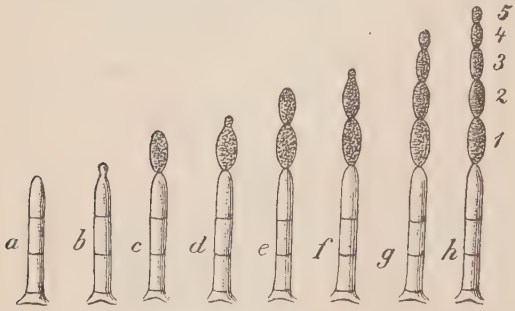


FIG. 104.—Diagrammatic sketch of the formation of conidia, according to type II.

For explanation see text. (*After Zopf.*)

When first formed, each conidium is unicellular; and in most fungi it remains so until germination occurs. On the other hand, in some species the interior of the spores becomes divided into compartments by the formation of one or more septa.

The production of conidia, especially those of the second type, will probably remind the reader of the process referred to as gemmation in § 219. In fact the sole morphological difference between the two processes is that the one occurs on *vegetative* organs and the other on organs of *fructification*. If this difference be obliterated and the mycelium itself, at a certain period of its existence, becomes an organ of fructification, then the choice of the terms, gemmating mycelium or conidial aggregation, for the resulting cells, becomes an arbitrary one. The term, "**yeast conidium**," occurring in the older literature, must be interpreted in this sense as mostly applying to budded cells articulated from *Hyphomycetes*, and capable, from their method of formation, of being regarded as conidia, as well as resembling in their (more or less ovoid) shape the majority of the yeasts. Such forms are found in the *Dematium pullulans*, to be described in the last section but one, and also in many other fungi.

Little attention has yet been devoted to the relation between the conditions of cultivation and the production of conidia. According to G. KLEBS (I.), in the case of *Eurotium* (*Aspergillus*) *repens*, the **motive force** is probably hyphal transpiration; and consequently a tendency to the production of spores is developed when the mycelium is constrained to exert a certain force in order to extract the water from the nutrient substratum. According to C. TANRET (I. and II.), *Aspergillus niger* will not produce conidia when obliged to develop, at 30°–40° C.,

in a Raulin's nutrient solution containing 0.5 gram, or more, ammonium nitrate per 100 c.c., or larger quantities of the sulphate or chloride of this base; whereas the process is stimulated by the presence of even as much as 2 grams of ammonium phosphate. Free acid, up to 0.4 gram per 100 c.c., is liberated from the first-named salts. At 20°–22° C., on the other hand, even as much as 1 gram of ammonium nitrate merely retards, without preventing, fructification. Experiments in the same direction have been made by W. Schostakowitsch (I.), especially with *Dematium pullulans*, as will be found in a later section relating to this organism. (*See also* § 229 and § 233.)

In some fungi the conidiophores, instead of remaining separate, arrange themselves in parallel order, side by side, at an early stage, and thus unite to a fascicle, to which the name **coremium** has been given. In a still higher stage of development the conidiophores—compressed together like a palisade—and the chains of conidia thereon, are enclosed in a cover; which latter is developed from the adjacent mycelium, may consist of one or more layers, and is closed on all sides when young, but afterwards opens to allow the ripened spores to escape. These forms are termed **pycnides**, an example of which is afforded by the æcidia of corn mildew, *Puccinia graminis*, which occurs in the form of red patches on barberry leaves, and, together with a number of similar parasites, constitutes the class of the *Uredineæ* or *rust fungi*. These latter, as well as the adjacent class of *Ustilagineæ* or *smut fungi*, do not come within the province of the present work, but are treated thoroughly in handbooks on plant diseases. Technical Mycology is concerned solely with certain genera of the *Zygomycetes* and *Ascomycetes*, the first-named being dealt with in chapters xliii.–xlv., and the others in succeeding chapters.

It was stated in § 220 that sporangia make their appearance in two different principal stages of development, and that the higher of the two differs from the other by greater precision as regards the number, form, and dimensions of the endospores, produced in the sporangium (or ascus). A similar distinction may also be made in connection with the formation of conidia. Among the *Eumycetes* there exists a well-defined group of fungi, in most species of which the conidiophores exhibit constancy in respect of their form and dimensions, as well as with regard to the number and form of the conidia they produce. This fact has been expressed by the bestowal of a particular nomenclature, such conidiophores being termed *basidia*, and the group of fungi **Basidiomycetes**. It includes most of the edible fungi (*e.g.*, the mushroom).

By introducing into the classification scheme laid down in § 220 the further subdivision based on the peculiarities enunciated above, we obtain the following:

Classification of the *Eumycetes*.			Class.
Eumycetes	*Mycomycetes* (septate mycelium)	(*a*) *not forming* endogenous spores	1. *Basidiomycetes* 2. *Uredineæ* 3. *Ustilagineæ*
		(*b*) *forming* endogenous spores	4. *Ascomycetes*
	Phycomycetes (aseptate mycelium)	(*a*) *forming* zygospores	5. *Zygomycetes*
		(*b*) *forming* oospores	6. *Oomycetes*
		(*c*) *forming* neither zygospores nor oospores	7. *Chytridiaceæ*

The statement that the **formation of endospores** is peculiar to only a single class of *Mycomycetes*, the *Ascomycetes*, must not, however, be taken to imply that the fungi of this class are exclusively reproduced by means of ascospores. On the contrary, emphasis must be given to the fact that the production of conidia also occurs in these fungi; hence this class is richer than the other three, as regards methods of fructification.

§ 223. The Formation of Oidia and Gemmæ.

It frequently happens that the mycelium proceeds direct to the formation of conidium-like cells, without first producing conidiophores. In such cases the whole or part of the mycelium subdivides into short pieces, which are capable of subsequently germinating and thus playing the part of spores. When the mycelium in question belongs to one of the *Phycomycetes*, it must, for this purpose, produce internally as many tranverse septa as will correspond with the number of resting cells to be formed. This constitutes the exceptional case referred to in § 218, where the occurrence of septa in the unicellular mycelium of a *Phycomycetes* can be observed under normal circumstances. Special partition walls are also formed in such cases in the already septated mycelium of the *Mycomycetes*. The formation of germinative resting cells by the breaking up of the mycelium was first observed in the case of a fungus known as *Oidium lactis*, which will be more fully considered in a later section. The generic name of this fungus is now employed to distinguish the resting cells in question, which are therefore called **oidia**. Their appearance is a very ordinary phenomenon throughout the entire realm of the fungi; and it not infrequently happens that the whole of the mycelium is affected, and undergoes conversion into a coherent chain of **oidia** (Fig. 105).

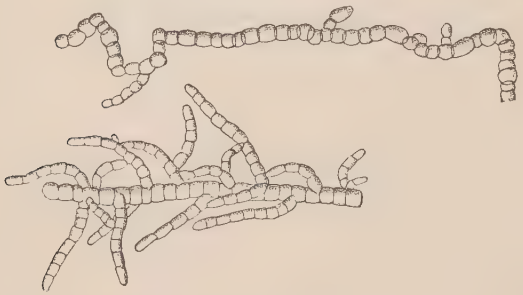


Fig. 105.—Chlamydomucor racemosus, Brefeld.

Fragment of mycelium which has undergone conversion into chains of oidia. Magn. 120. (*After Brefeld.*)

In some instances this articulation and formation of germinative cells goes a step farther in order to ensure the attainment of its purpose, inasmuch as the cells are formed into veritable **resting cells**, whereas, as a rule, the oidia cannot be characterised as such, their powers of resistance being no greater than those of the mycelium from which they are elaborated. The conversion into resting cells may be effected by the thickening of the membrane of the oidia, and the absorption of nutrient material from the adjacent portions of the mycelial plasma, in consequence of which the cell in question increases in size. Owing to the large fat-content of these cells, they strongly refract light, and thus show up with particular lustre (like a cut precious stone (gemma) in a necklace) against the exhausted adjoining portions of the hypha. Such a cell is termed a **gemma**, **chlamydospore**, or **brood cell**. They are more usually formed in the mycelial hyphæ, but occasionally also appear on fruit-bearing stems, which have either fulfilled their true purpose or are in the act of so doing. An example of both instances is shown in Fig. 106. In each case the gemmæ have been produced within the hypha, or conidium, and are therefore **intercalary**; though instances are known where the gemma is formed on the crown of an erect hypha, on which it is supported as by a stem, and is therefore known as a **peduncular gemma**. The first observation of the production of chlamydospores was made in 1885 by Caspary (I.), who recognised them as a new method of fructification and gave them the name **arthrospores**, which, however, is no longer used in this sense (§ 55).

With regard to the arbitrary **production** or **suppression** of gemmæ, certain

experiments were made on *Mortierella van Tieghemii* by J. Bachmann (II.), who found that, in cultures on a solid nutrient substratum, concentration of the medium restricts the production of sporangia and finally suppresses same, the formation of peduncular gemmæ being correspondingly increased.

The gemmæ should not be confounded with mere swellings of the mycelium, the latter being distinguishable, in the first place, by the absence of the terminal septa and definite shape, and making their appearance as a result of unfavourable conditions of existence. Lopriore found them on the germinative tubes of *Mucor mucedo* — which does not produce gemmæ at all—when the spores of this organism (placed in a suitable nutrient solution) were exposed to an atmosphere containing 60 per cent. of oxygen and 40 per cent. of carbon dioxide. Eschenhagen (I.) observed similar malformations in cultures grown in excessively rich nutrient solutions, *e.g.*, a 60 per cent. sugar solution. According to M. O. Reinhardt (I.) they also occur in mixed cultures, as a result of the injurious effect, on one of the symbiotic organisms, of the metabolic products of the other.

Fig. 106.—Chlamydomucor racemosus, Brefeld.

On the right is a fragment of a mycelial hypha with six chlamydospores. On the left a sporangium stem with five chlamydospores; on the crown of the columella are a few endospores together with the remainder of the sporangial membrane, the bulk of which has been removed during the preparation of the specimen. Magn. 80. (*After Brefeld.*)

A fungus is said to be **monomorphous** when the same is only known to fructify in one single manner; whereas fungi exhibiting two or more methods of fructification are termed **pleomorphous.** The *Mucorineæ* (§ 235) form a good example of pleomorphism, and the same occurs in the, to us, still more interesting *Saccharomycetes*, which latter always exhibit three different methods of fructification, viz., by conidia, gemmæ, and endospores. Owing to their Protean character, these organisms occupy a peculiar position in the general morphology of the fungi; and in them we see, more than elsewhere, how the same organ can change its nature and undergo modification from one to another. According to the comprehensive researches of Hansen (XXVIII.), the yeast cell may serve (1) as a conidium, for vegetative reproduction; (2) as part of a mycelium; or (3) as an ascus, and therefore producing internal spores. Finally (4), the spore is capable of not only acting vegetatively (germinating), but also, under certain conditions, of becoming an ascus by forming spores (of the second order) in its interior. Compare § 248.

Among the *Eumycetes*, the oidia and gemmæ are the most frequent varieties of fruit; but this circumstance, coupled with the simplicity and uniformity of their structure, renders these characteristics almost entirely valueless as a basis of classification for the fungi, since this classification is mainly founded on differences in the occurrence, mode of production, and development of one or more of the other three reproductive organs (endospores, conidia, zygospores), in the species to be differentiated. Fungi wherein these organs have not yet been observed, or wherein the latter appear in a form which does not permit their inclusion in the existing system, are set apart in a special class as "**Fungi imperfecti**," a term, however, expressing not incompleteness in the fungi them-

selves, but only in our knowledge concerning them. A few of these species, *e.g.*, the so-called *Saccharomyces apiculatus*, certain *Mycodermas*, the *Moniliæ*, the *Torulæ*, &c., fall within the province of the present work, and will be dealt with fully in the final section.

§ 224.—The Germination of Spores. Their Tenacity of Life.

The spore is ripe when it has acquired the capacity of developing into a new individual of the species from which it originated. The first stage of this

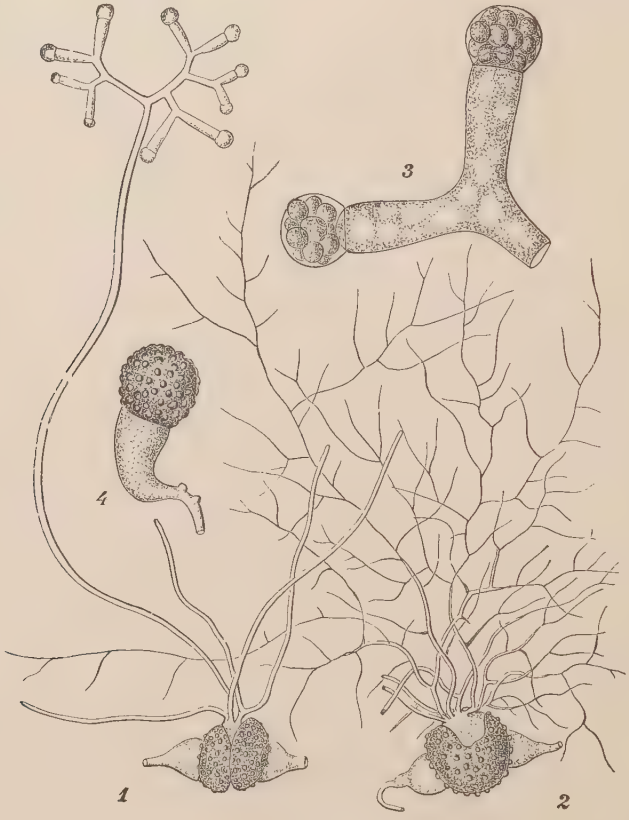


FIG. 107.—Sporodinia Aspergillus, Schrank.

1. Fructificatively germina ing zygospore. Magn. 40. (*After Brefeld.*)
2. Vegetatively germinating zygospore. " "
3. Two ripe sporangia of this fruit-bearing stem. Magn. 150. (*After De Bary.*)
4. Azygospore. Magn. 90. (*After F. von Tavel.*)

development is termed **germination**, and, in the case of endospores and conidia, is a comparatively simple process. It has already been fully discussed in § 218, and illustrated in Figs. 92 and 93. Deviations from the main lines there laid down, however, sometimes occur; and with one of these we shall later on become acquainted in the case of *Saccharomyces Ludwigii*. While previous separation from the parent plant is unnecessary for the commencement of germination in the conidia, the endospores must have been set free therefrom

by the decomposition or breaking down of the wall of the sporangium or ascus. More will be said on this point in § 235. Special experiments by P. Lesage (III.) have placed beyond doubt that the development to mycelium of a spore germinating on a solid nutrient substratum proceeds the more rapidly and luxuriantly the higher the water-vapour tension of the superincumbent air.

The germination of the zygospores commences by the bursting of the episporium, and the extrusion of the endosporium, in one or more places, by the pressure of the swelling cell contents. When the zygospore is submerged in a liquid, the development is of a vegetative character, a mycelium being produced; but, when, on the other hand, the spore is exposed to the air, it puts

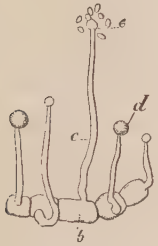


Fig. 108.—Chlamydomucor racemosus, Brefeld.

Chain of five chlamydospores, each of which has put forth a sporangiophore. The sporangium of *d* is still unripe; whilst that in *c* is already destroyed, only the columella (*e*) and eight endospores being left. Magn. 120. (*After Brefeld.*)

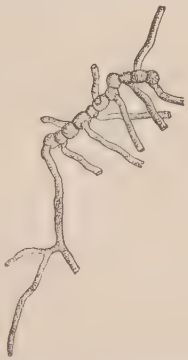


Fig. 109.—Chlamydomucor racemosus, Brefeld.

Vegetative germination of a chain of eleven oidia. Magn. 80. (*After Brefeld.*)

forth (fructificatively) a fruit-bearing stem, which then produces spores in its turn. An example of both instances, in zygospores of one and the same species, is given in Fig. 107. *See also* division 5 in Fig. 101.

The same also applies, in many instances, to chlamydospores, *i.e.*, these also may germinate in two ways: either vegetatively or fructificatively. An example of a fungus possessing these properties is afforded by *Chlamydomucor racemosus*. The fructificative germination of its chlamydospores is represented in Fig. 108. The vegetative germination of these resting cells was first observed by Th. Bail (I.) in 1857, on species of *Mucor*, this worker at the same time making the important discovery that the chlamydospores, when submerged, develop to a budding mycelium, and not to the typical form.

In most cases the germination of the oidia is purely vegetative; it is illustrated in Fig. 109. In many fungi, certain *Mucorineæ* in particular, they are, however, also capable of fructificative germination when placed under the same conditions as the chlamydospores.

Tenacity of life, *i.e.*, capacity for withstanding adverse conditions, is generally higher in spores than in the corresponding mycelia. Bibliographical collections on this point are given by A. de Bary (I.), W. Zopf (X.), W. Pfeffer (III.), and others; but only a few instances can be mentioned here. With regard to resistance towards drought and desiccation, the premier place, so far as is yet known, is occupied by the *Anixiopsis stercoraria* discovered and investigated by E. Chr. Hansen (XXVII.). The ascospores of this congener of ordinary bread mould proved still capable of germination after storage in a dry state for twenty-one years. The same observer also found life retained by the conidia of *Aspergillus glaucus* after sixteen years' quiescence, and by those of *Aspergillus flavescens*

for eight years (but not longer); whilst Eidam (III.) found the conidia of *Aspergillus fumigatus* alive after ten years; Brefeld (IV.) those of *Aspergillus flavus* after six years; C. Wehmer (IX.) those of *Aspergillus oryzae* after more than four years, those of *Aspergillus niger* after about three years, and of *Aspergillus Wentii* after more than a year. Of course these findings do not apply to each individual spore of the species in question, the weaker specimens dying off at far earlier periods than those mentioned. With regard to the resisting powers of the ascospores of *Saccharomycetes*, and the practical consequences thereof, more will have to be said in a later chapter. In the case of many fungi, the resting cells must remain quiescent for a certain period before they become capable of germinating. This applies particularly to the whole of the zygospores and oospores. On prolonged exposure to the air the contents of the spores gradually dry up, so that the membrane soon becomes wrinkled; but immersion in water causes them to swell out and resume their plumpness.

The **power of resisting heat** varies according as the heat acts on the fungus spores in the dry state (§ 76) or in presence of moisture; in the latter event they die off much more readily and speedily. Thus, it was shown by Pasteur that the conidia of *Penicillium glaucum* perish when diffused in a liquid at 100° C., whereas when moisture is excluded they will resist a temperature of 120° C. for some time, though incapable of standing 127° to 130° C. Further particulars under this head will be given in the paragraphs dealing with the moulds growing on bread, &c., in a later section.

Divergent opinions have been expressed as to the cause of the high resistance presented to dry heat by the spores of *Eumycetes*. E. Cramer (III. and IV.), on the basis of certain chemical analyses, ascribes this property to the high concentration of the cell contents, the residual water being insufficient in quantity to allow of the coagulation of the albuminoids. He found, for instance, about 61 per cent. of dry residue in the spores, but only about 12 per cent. in the corresponding mycelium; though in the former the ash content (3.1 per cent.) was smaller than in the latter (11.3 per cent.). In view of the already emphasised dependence of the chemical composition of the micro-organisms (§ 36) on the nature of the nutrient substratum, it is a somewhat risky matter to draw general conclusions from such analytical data. This also applies, be it remarked, to the results of the chemical analyses of the mycelia of *Rhizopus nigricans*, *Penicillium glaucum*, and *Aspergillus niger* (*glaucus?*) made by Marschall (I.).

In respect of **resistance to low temperatures**, what has already been stated in § 61 applies also, in the main, to the spores of *Eumycetes*. On the other hand, the mycelia, especially when rich in sap, frequently perish at a few degrees below zero C.; *e.g.*, according to Molisch (V.), that of *Phycomyces nitens*. Others again, such as pressed yeast, which is poor in water, will stand very low temperatures.

As regards the resistance of the spores of *Eumycetes* towards poisons, the observations already recorded in vol. i. may be supplemented by reference to the works of O. Löw (II.), B. Joensson (I.), and F. L. Stevens (I.).

We have now learned, in connection with the general morphology of the *Eumycetes*, everything necessary to enable the following chapter to be readily intelligible. In the event of any reader desiring to learn more with regard to the structure of fungi in general, the handbook compiled by W. Zopf (X.) can be consulted, after which he may profitably study the work of A. de Bary (I.), which still—twenty-five years after its first appearance—remains the standard work on mycology. The results of the labours of O. Brefeld, which in many respects were based on other fundamental views, are contained in a work published by F. von Tavel (I.).

With regard to all concerning the **general physiology** and **biology** of the *Eumycetes*, the reader can rely on the first of the three books named. During

the period of nearly two decades that has elapsed since it was completed, the subject has made considerable advances in five different directions, viz., the chemistry of the cell membrane, the requirement of mineral nutrient substances, the stimulative influence of light, chemotropism, and the production of enzymes. These questions alone will be treated in the three following chapters of the present work, since it is no part of the author's purpose to supplant existing good books. Furthermore, the reader will quickly see that there has been no stringent limitation to the subjects cited, but that every opportunity has been taken in order to gain a general view during the necessary consideration of particular points. When presented in conjunction with the treatment of questions possessing visibly practical importance, certain general explanations will be more readily appreciated by the reader than if given at the commencement of the book, where they would either pass unnoticed or fail to interest.

CHAPTER XL.

CHEMICAL COMPOSITION OF THE CELL MEMBRANE OF EUMYCETES.

§ 225.—Cellulose.

The question as to the **chemical composition** of the substance constituting the **cell membrane of fungi** could not be agitated until some information had been gleaned in connection with the anatomical structure of these organisms. This condition was still unfulfilled at the time (1811) when H. Braconnot (II.) subjected certain edible fungi to lixiviation, and applied the named **fongine** to the residue obtained in this manner. Certainly this substance, the name of which was afterwards Latinised to **fungine**, was not a uniform body; nor does the circumstance that it was then new justify the assumption that Braconnot was the first to prepare the cell membrane of fungi in a pure state. During the fifties, Payen subjected various fungi to lixiviation with ether, alcohol, alkalis, and acids, and made ultimate analyses of the residues. In this manner he obtained figures which fairly corresponded to the formula $C_6H_{10}O_5$. Being unaware of the immense number of isomeric substances, all possessing this elementary formula, he deduced from his data the occurrence of pure cellulose in the fungi, and denied the existence of the alleged fungine. The same conclusions were formed in succession by Schlossberger and Döpping (I.), as also by Fromberg—whose researches were reported by Mulder (IV.)—and by A. Kaiser (I.) in 1862.

This hypothesis of the identity of the substance composing the cell membrane of fungi on the one hand, and that of higher plants on the other, was based more on prejudice than on the results of analysis, and necessarily fell to the ground on the discovery of various reagents giving peculiar and distinctive reactions with true cellulose: for instance, ammoniacal copper oxide, which was introduced by E. Schweizer (I.) in 1857, and dissolves pure cellulose without leaving any residue; zinc iodochloride, which was recommended by C. Nägeli (VII.) and H. von Mohl (I.), and gives a violet coloration; and finally iodosulphuric acid, which gives a blue coloration and was first employed by Schleiden. Soon after the introduction of the first-named reagent, Fremy (I.) showed that, in many cases (*e.g.*, mushrooms), it is incapable of dissolving the cell membrane of fungi, and, consequently, that the membrane is constructed, not of pure cellulose but of a substance (of unknown constitution) to which he gave the name metacellulose. A similar opinion on this point was pronounced by A. de Bary (III.), who gave expression to his views by employing the name **fungus-cellulose** for the substance in question. In this connection mention should also be made of Mulder's observations on the behaviour of the membrane of yeast towards iodosulphuric acid. More complete particulars with regard to this class of cell membrane will be found in § 249.

Very soon, however, doubt began to arise as to whether any special or peculiar character was really possessed by the so-called fungus cellulose. As far back as 1858 it was shown, by Carl Cramer (I.), that the solvent action of Schweizer's reagent on true cellulose was retarded, or even entirely prevented, by the presence of foreign incrustations in the membrane. Opinion then showed a tendency to

favour the idea that the membrane of fungus cells contains a fundamental substance like cellulose—and to which Tschirch (III.) gave the name **mycin**—but that this could not be detected by the aforesaid reagents owing to the presence of interspersed incrustations. This view was specially championed by K. Richter (I.). By his investigations on a series of fungi—including *Secale cornutum*, *Agaricus campestris*, and others wherein A. de Bary had observed the assumed fungus cellulose—this worker, in 1881, proved that when such membranes did not immediately give the reactions in question (especially with iodine), they could be induced to do so by steeping them for not less than a fortnight in a 7 to 8 per cent. solution of caustic potash. The elementary composition of the membrane so treated, he found to correspond to the formula $n(C_6H_{10}O_5)$.

Even this observation did not remain unopposed, the same hypothesis being urged against it that every investigation into the nature of the cell membrane has had to contend with ever since the days of Payen, namely, the question whether this preliminary treatment of the membrane merely results in the extraction of the extraneous admixtures, or whether it is not rather that the fungus cellulose is converted into true cellulose. However, even apart from this doubt—which will be further considered in subsequent paragraphs—Richter's observations do not disprove the assumption that a substance, differing from pure cellulose, occurs in the membrane of fungi, since, in some of his experiments, the cellulose reaction could not be observed in the preparations employed. Nevertheless, although in view of the last-named circumstance it cannot be admitted that, strictly speaking, Richter's experiments actually identified fungus cellulose with true cellulose, it is justifiable to assume from his results that pure cellulose really does occur in the cell membrane of fungi. An attempt to support this assumption was made by I. Dreyfuss (I.) in 1893. For the purpose of extracting the cell contents and obtaining the pure membrane substance, this worker employed the method given by Hoppe-Seyler (III.). This consisted in treating the specimen with alcohol, ether, water, 2 per cent. hydrochloric acid, and 2 per cent. caustic soda, and then heating to 180° C. with concentrated caustic potash, whereby—as was then supposed—everything except pure cellulose was decomposed and dissolved. After acidifying the cooled melt the residue could then be separated by filtration, and tested for cellulose by the reagents already specified. In addition to cultures of bacteria, Dreyfuss examined certain higher fungi, viz., *Agaricus campestris*, *Polyporus officinalis*, and *Aspergillus glaucus*; and apparently found cellulose in all of them.

In view of the greater insight since afforded into the multiplicity of the isomers and analogues of cellulose, and of which the earlier workers had not the slightest idea, the successors of Richter in this branch have found it desirable to enlarge the limits of the question. Instead of the conflict respecting the identity of fungus-cellulose and true cellulose, there arose the endeavour to identify the various constituents of which the membrane is presumably constructed; and the search for cellulose was relegated to a subsidiary position. For more comprehensive investigations in this respect we are indebted in the first place to C. van Wisselingh (I.), who, in 1898, examined no less than about a hundred species, comprising members of nearly all the orders, and most of the families, constituting the fungoid kingdom. For extracting the samples he substituted for the above method a new one devised by himself, namely, heating the fungus in glycerin, contained in a sealed tube immersed in an oil bath, and raised to 300° C. within half an hour. By this means the extraction is so far advanced that the residue can be tested for cellulose without opening the door to any objection. Apart from the *Myxomycetes*, with which we are not concerned, Wisselingh could only detect the presence of cellulose with certainty in two families of fungi, namely, the *Saprolegniæ* and the *Peronosporeæ*, both

belonging to the *Oomycetes* (*Phycomycetes*, *see* § 220). On the other hand, this carbohydrate could not be detected either in the *Zygomycetes* or in any of the *Mycomycetes* examined: more particularly not in *Saccharomyces cerevisiæ*. These results agree with those obtained by E. Gilson (I.) in the examination of *Mucor vulgaris*, *Thamnidium vulgare*, and *Agaricus campestris*. The contradictions in the results of Richter and Dreyfuss will be explained in the next paragraph. Substances possibly allied to cellulose, since they have many reactions in common therewith, were observed by Wisselingh in the fungus of the lichen known as *Usnea barbata*, and in *Geaster fornicatus* (a fungus allied to the well-known *Bovistes*), on which account he named them **usnein** or **geasterin**. On the other hand, the substance which Mangin (I.) classed as cellulose in the case of *Mucorineæ*, *Uredineæ*, and *Ustilagineæ*, does not –according to his own reports—give the typical reactions, and hence cannot lay any claim to recognition.

It being thus certain that true cellulose has, so far, been found in only two families of fungi, it now remains to ascertain the composition of the cell membrane of the others. Thanks to the zeal of several workers, whom we shall mention shortly, satisfactory, and perhaps even surprising, information can already be given on this point; for it will undoubtedly seem strange to hear that one of the components in question is nitrogenous. This is positively known, and the substance in question will be dealt with in the following paragraphs as being most worthy our attention. Before dealing with this, however, it is advisable to state, in order to prevent misunderstanding, that the workers in question mostly employ the term fungus-cellulose in the sense of fungus membrane substance.

§ 226.—Chitin.

That the residual substance of the cell membrane, after passing through various methods of extraction, always contained nitrogen, was recognised not only by investigators—such as Schlossberger and Döpping (I.)—in an age of imperfect analytical knowledge, but also more recently by W. Hofmeister (I.) and E. Winterstein (I.). The last-named found **nitrogen** (3.3 to 3.9 per cent.) in preparations from the cell walls of the edible boletus (*Boletus edulis*); 3.6 per cent. in the case of the mushroom (*Agaricus campestris*); 3 per cent. in the toadstool (*Cantharellus cibarius*); 2.5 per cent. in the common morel (*Morchella esculenta*); 3.3 per cent. in *Penicillium glaucum*, and 3.9 per cent. in a species of *Botrytis*. Unlike his predecessors, however, Winterstein did not rest content with the comforting belief that the extraction of the nitrogenous matter in the cell membrane had not been effected completely, but convinced himself that nitrogen was also present in such preparations as had been perfectly freed from nuclein and albumen by the previous treatment with caustic potash and Schulze's reagent. It therefore followed that the preparations in question must be partly or entirely composed of some nitrogenous substance. The same discovery, in the main, was obtained independently a few months later by Eug. Gilson (II.) in his researches on *Agaricus campestris* and ergot of rye (*Secale cornutum*). By fusing the cell preparations therefrom with caustic potash at 180° C., according to the Hoppe-Seyler method, he obtained a residue, not of cellulose but of a substance insoluble in Schweizer's reagent, and to which he gave the name **mycosin**, the elementary composition being, moreover, ascertained to correspond with the formula $C_{14}H_{28}N_2O_{10}$. This substance is a base, the chloride of which is thrown down when concentrated hydrochloric acid is added to the aqueous solution. Mycosin itself is soluble in 2 to 3 per cent. hydrochloric acid or in very dilute acetic acid. A solution of iodine in potassium iodide, containing a trace of free acid, gives a reddish violet stain. According to Wisselingh (I.) and E. Zander (I.), zinc-iodochloride solution varies in action in accordance with

the amount of zinc chloride present, 50 per cent. producing a blue to blue-violet coloration. These last reactions closely resemble those of cellulose, and might easily be mistaken by an inexperienced observer as indicating the presence of that substance. This possibility will be referred to later on.

A closer indication of the position to be allocated to mycosin in the immense field of organic nitrogen compounds was afforded in a second investigation of WINTERSTEIN'S (II.) by the discovery that this substance is decomposed, on hydrolysis with 3 per cent. sulphuric acid, into: *d*-glucose as the chief product; then probably other hexoses as well, and then into acetic acid and an undetermined nitrogenous organic substance. Decisive conclusion as to the method of combination of the nitrogen in this cell-membrane substance was soon afterwards furnished by WINTERSTEIN (III.) through the discovery that, when heated with concentrated hydrochloric acid, it yielded a crystallisable fission product, which proved identical with the hydrochloride of chitosamine, $C_6H_{11}O_5.NH_2.HCl$—at that time erroneously termed glucosamine, even by Winterstein. As Winterstein at once recollected, the same behaviour is exhibited by **chitin**, *i.e.*, the substance (discovered by Odier) composing the chiton and elytra of insects, spiders, crabs, and other lower animals. This substance, as was shown by G. LEDDERHOSE (I.) in 1876, furnishes under similar treatment the "**glucosamine**" discovered by that observer; and that too in the state of hydrochloride, from which latter the free base, *i.e.*, **chitosamine** itself, $HO.CH_2-(CHOH)_3-CH.NH_2-CHO$, was first isolated by C. A. Lobry de Bruyn. This led to the unexpected conclusion that the cell membrane of the fungi (under examination) was to a greater or smaller extent constructed of chitin. If this should be the case, then the said preparations of the cell membrane must also exhibit the highly characteristic reaction for chitin, discovered by HOPPE-SEYLER (II.), namely, the dissociation of this substance into acetic acid and **chitosan** by exposure to fusible caustic potash at 180° C. Chitosan is basic in character, and is precipitated from its acetic solution by an excess of caustic potash. The chloride is insoluble in strong hydrochloric acid. A similar dissociation product is already known in the mycosin prepared by Gilson in 1894; and, as a matter of fact, chitosan and mycosin are identical, and can be isolated by the same method, both from animal chitin and the cell membrane of fungi. In this manner it was proved that chitin is also present in the fungoid kingdom, and is not, as was formerly supposed, of exclusively animal origin; and indeed the proof was strengthened by E. GILSON (III.) and E. WINTERSTEIN (IV.) isolating chitin, as such, from *Agaricus campestris*. The nitrogen content of this substance was determined by these workers as 6.24 per cent., a value agreeing fairly well with the 6.01 per cent. calculated from the formula ($C_{18}H_{30}N_2O_{12}$) set up for animal chitin by G. STÄDELER (I.) and confirmed by T. ARAKI (I.) in 1895. On the basis of this formula the two hydrolytic reactions above referred to may be expressed by the equations:

$$\underset{\text{Chitin}}{C_{18}H_{30}N_2O_{12}} + \underset{\text{in the potash}}{2H_2O} = \underset{\text{Chitosan}}{C_{14}H_{26}N_2O_{10}} + \underset{\text{Acetic acid}}{2C_2H_4O_2}.$$

$$\underset{\text{Chitosan}}{C_{14}H_{26}N_2O_{10}} + \underset{\text{in the conc. HCl.}}{2H_2O} = \underset{\text{Chitosamine}}{2C_6H_{13}NO_5} + \underset{\text{Acetic acid}}{C_2H_4O_2}.$$

It may be mentioned that chitosamine, and even chitin, can be split up by the prolonged action of concentrated hydrochloric acid into ammonia and a sugar:

$$C_6H_{11}O_5.NH_2 + H_2O = NH_3 + C_6H_{12}O_6,$$

to which Berthelot has given the name **chitose**. The formation of this sugar by

the action of concentrated hydrochloric acid on preparations of the cell membrane of fungi has already been observed by Städeler (I.) without, however, the nature of the reaction having been recognised.

Bearing in mind the fact that Dreyfuss employed concentrated potash at 180° C., under which circumstances the chitin present would be converted into chitosan, the latter—according to Gilson—then behaving like cellulose in presence of certain reagents, it will be readily understood why Dreyfuss arrived at the conclusion that he had to deal with pure cellulose. According to the results obtained by Wisselingh (I.), the same conversion, and consequently the same liability to deception, also occurs when chitin is exposed for a considerable time to the action of dilute (7 to 8 per cent.) caustic potash at the ordinary temperature, *i.e.*, the same treatment as employed by Richter in purifying the cell membrane preparations.

Finally, it is not inappropriate to recall that the formula given above for chitin is not yet entirely beyond dispute. It cannot at present be definitely stated whether the relative values of the bodies taking part in the foregoing reactions are accurately represented by the equations laid down by Araki; in fact we have still to face the possibility of there being a whole series of isomeric or nearly allied substances, and therefore of the necessity of regarding the name chitin as a collective term.

The extent to which chitin is present in different groups of fungi was investigated by C. van Wisselingh (I.) in researches already referred to in previous paragraphs. Its absence was confirmed by this worker in the case of *Bacteria*, *Oomycetes* (*Saprolegnieæ* and *Peronosporeæ*) and *Saccharomycetes* examined by him. In all the other species of fungi examined, however (about a hundred), the presence of chitin was invariably detected, *e.g.*, in *Mucor mucedo*, *M. racemosus*, *Rhizopus nigricans*, *Penicillium glaucum*, *Tricothecium roseum*, in the sclerotia of *Botrytis cinerea* and *Claviceps purpurea*, &c. At present no instance of the simultaneous occurrence of cellulose and chitin in the cell membrane of a fungus is known. In many cases, *e.g.*, the perithecial wall and the asci of *Aspergillus glaucus*, the membrane is partly composed of other substances. With the assistance of improved microchemical tests Wisselingh has also studied the local distribution of chitin in the cell membrane, and has found, for instance in the wall of the ascospores of *Aspergillus glaucus*, that chitin is present in the form of two kinds of biconvex plates.

For information respecting the amount of chitin present in certain fungi we are indebted to C. Tanret (III.), according to whose determinations—which, however, must be regarded as merely approximate estimates—about 15 per cent. of this substance is found in *Aspergillus niger*.

§ 227.—Hemicelluloses and other Carbohydrates.

Of the two main groups of carbohydrates of the formula $C_6H_{10}O_5$ entering into the composition of cell membranes, the occurrence of true cellulose in fungi has been dealt with in § 225, and there remain therefore only the representatives of the second group, namely, the **hemicelluloses** (§ 118) and other easily hydrolysed carbohydrates. Apart from the report furnished by Braconnet (I.), we are indebted to the French chemist Champignon (I.) for the earliest observation of these substances. From a subterranean fungus, *Pachyma Cocos Fries*, largely consumed in China under the names Fuh-ling, Fouh-ling, and Pe-fuh-ling, this worker isolated a carbohydrate of the formula $C_{30}H_{48}O_{28}$, which, on being treated with dilute acid, is converted into a liquid capable of reducing Fehling's solution. To this carbohydrate he gave the name **pachyman**. Later, E. Winterstein (II.) isolated from the edible boletus (*Boletus edulis*) a carbohydrate which is soluble

in dilute acids and reprecipitated by alcohol. This substance has received the name **paradextran**, and its elementary formula is $C_6H_{10}O_5$. Closely allied thereto, and of the same composition, is **paraisodextran**, which is turned blue by iodo-sulphuric acid and has been detected in several fungi by E. Winterstein (IV.). According to an observation made by the same worker (III.), **true hemicellulose**, in the sense applied to the term by E. Schulze, also seems to occur in fungi. Two mucinous carbohydrates, known as **mycetide** and **viscosin**, but not yet closely examined, are found in various mushrooms, according to a report of Boudier's cited by A. and Th. Husemann (I.). The former of these substances presumably resembles the gums, whilst the other probably is closely allied, chemically speaking, to the mucilage found in linseed and *Plantago Psyllium L.* According to an analysis made by J. L. Keller and reported by the Husemanns, **pectose** has been isolated from Fouh-ling.

Still undetermined is the chemical character of the substance giving the blue iodine reaction observed by Friedrich Hoffmann and by Paul Lindner (VIII.) in the case of the phlegmated hyphæ of *Dematium pullulans* and the membrane of the spores and sporogenic cells of *Schizosaccharomyces octosporus*, and also by E. Cramer (III.) on the conidia of *Penicillium glaucum*. The substance in question is certainly not starch, but is possibly an isolichenin. Moreover, the blue reaction with iodine solution is by no means a rarity, being observed, for example, on the apices of the asci of many *Pyrenomycetes* and *Discomycetes;* whilst similar observations have been made by O. E. R. Zimmermann (II.) in different species of *Mucor*. The red mould (of the genus *Fusarium*) occurring as red spots and stripes on barley and malt, where it was first observed by C. G. Matthews (I.) and afterwards by Carl Klein (I.), excretes—through the swelling of the outer layer of the cell membrane—a mucilage which is coloured violet by iodine.

After employing the usual treatment for the determination of crude fibre, C. Tanret (I.) found, in the thallus of *Aspergillus niger*, the hard mycelium of *Claviceps purpurea*, the corpus of *Polyporus officinalis* and *Boletus edulis*, and in yeast, a carbohydrate which he named **fongose** and to which he ascribed the formula $(C_6H_{10}O_5)_6$.

The presence of a **carbohydrate of the pentosan group** ($C_5H_8O_4$) has been detected in several species of fungi by Dreyfuss (I.). The cell membrane preparations made by Winterstein (II.) must also have contained pentosans, since they furnished 1 to 2 per cent. of furfurol when distilled with hydrochloric acid.

Basing on the results of microchemical reactions performed with various colouring-matters, Mangin (IV.) thought himself justified in assuming that **callose**—a carbohydrate allied to the pectins and discovered by him in different phanerogams—also occurs, and indeed forms the chief constituent material in the cell membrane of fungi, and of the *Ascomycetes* in particular. At that time nothing was known regarding the presence of chitin in fungi; as, however, this latter substance behaves like the former in presence of several of the colouring-matters employed by Mangin, Wisselingh (I.) thinks it not improbable that the two might be confounded.

From Winterstein's report that the fungoid cell membrane preparations examined by him did not contain more than 3.9 per cent. of nitrogen, it must be concluded that these membranes were not exclusively composed of chitin, which contains over 6 per cent. of that element, but that considerable quantities of other compounds, poorer in or altogether devoid of nitrogen, were also present.

At the present time there is very little that is reliable known with regard to the local distribution of the constituents composing the cell membranes of fungi. Not that this deficiency is due to any lack of attempts to obtain information on

this point; on the contrary, these date as far back as the efforts made by W. Füisting (I.) in 1868. At a later period the matter was energetically taken up by L. Mangin (II.), who reported that, in the *Mucorineæ* examined by him, the inner layer of the septa and aerial hyphæ consists of cellulose, whilst the outer layer is composed of **pectin bodies.** Unfortunately the above-mentioned observations of Wisselingh have seriously called in question the reliability of the microchemical reactions on which Mangin based his assumptions.

The reader will not expect to find here any general reports on the thickness of the cell membrane in fungi. Nevertheless, mention may be made, in this connection, of a fact determined by Fr. Eschenhagen (I.), namely, that the concentration of the nutrient solution has a direct influence on the thickness of the cell membrane of the organism grown therein.

The cell membrane of fungi also often exhibits in a high degree the capacity of swelling; which, indeed, is frequently an indispensable faculty, especially in *sporangia* and *asci*, as being the only manner in which the endospores can be set at liberty. It is, moreover, not infrequently found in the case of vegetative cells, and will be dealt with in the paragraphs on the cell membrane of yeast. Where mucination of this kind is encountered one may reasonably assume the presence of pectin substances in the cell or membrane.

According to the concordant results obtained by A. Burgerstein (I)., M. Nigel (I.), and C. O. Harz (II.), lignification of the membrane does not seem to occur in *Mucor mucedo*, *Penicillium glaucum*, *Aspergillus glaucus*, and *Saccharomyces cerevisiæ*, or, according to the observations of the last-named worker, in *Mucor nigricans*, *Aspergillus conoideus Spreng.*, *Asp. candidus Link.*, *Asp. flavescens Rob.*, *Cephalothecium roseum*, *Tuber cibarium*, *T. æstivum*, *Claviceps purpurea*, and *Torula*. On the other hand, the lignin reaction (with sulphuric aniline sulphate) was furnished by the large pileated fungi, though whether the presence of lignin is thereby proved must remain an open question.

Not infrequently a deposition of colouring-matter is found in the cell membrane, usually in such a condition as to be incapable of extraction by any known solvent; this is the case with the conidia of *Aspergillus* and *Penicillium*. In other coloured fungi the colouring-matter is embedded in the plasma; of this a fine and technically important example has already been given in § 219.

Whether the waterproof character of some cell membranes, *e.g.*, in the conidia of *Penicillium* and *Aspergillus*, should be attributed to the deposition of **excreted fatty or waxy substances**, must be left undetermined. Biologically this phenomenon is important since it prevents the penetration of toxic substances from the surrounding aqueous medium, and thereby also opposes the attempts of the mycologist to kill such fungi by means of aqueous toxic solutions.

Deposits and incrustations of **calcium oxalate crystals** are of very frequent occurrence in the membrane of fungi, especially on the surface of the spores. In many cases their presence and appearance afford characteristic indications valuable for the purposes of classification.

CHAPTER XLI.

MINERAL NUTRIENT MATERIALS.

§ 228.—Alkalis.

A GLANCE through the existing analytical data concerning the **ash constituents of fungi** (*see* for example the handbook by J. KÖNIG (I.)) will soon reveal that the chief of these constituents are **phosphoric acid** and **potash.** The latter seldom forms less than one-quarter, and is generally about one-half of the total weight of ash, sometimes even rather more; the ash of truffles, for instance, according to an analysis by LIEBIG (II.), contains 54.5 per cent. of K_2O and 33.0 per cent. of P_2O_5. From this circumstance alone it may be concluded that, as has already been placed beyond doubt in the case of green plants, **potassium** is also of importance to fungi. The first experiment made in order to clear up this question was instituted by NÄGELI (IV.), and the results obtained led this worker to assume that potassium is so far non-essential to the growth of fungi that it can be replaced by **rubidium** or **cæsium**; but that one or other of these three must invariably be present.

With regard to cæsium, all subsequent investigators, however, agree that this metal is unsuitable for replacing potassium for the purpose in question. Instances of this are furnished by S. WINOGRADSKY (XI.) in his culture experiments with *Mycoderma vini*; W. BENECKE (II.) in the case of *Penicillium glaucum* and *Aspergillus niger*; and subsequently by E. GÜNTHER (I.) for *Mucor corymbifer*, *Rhizopus nigricans*, and *Botrytis cinerea*.

Opinions are divided as to the suitability of rubidium for replacing potassium. An affirmative result was furnished by O. LÖW's (VII.) culture experiments with a species of *Penicillium*, and by Winogradsky with the film-fungi already mentioned. On the other hand, in the experiments of W. BENECKE (II.) potassium was found replaceable by this allied metal, but only in cases where merely vegetative development was in question. Finally, the experiments of E. GÜNTHER (I.) furnished no uniform results: the cultivation in solutions containing rubidium, but no potassium, being successful in the case of *Botrytis cinerea*, but not so with *Rhizopus nigricans*. Now, in order to rightly appreciate these results it will be necessary to bear in mind the great difficulty experienced in completely freeing the rubidium salt from the accompanying potassium salts. This difficulty is, moreover, accentuated by the comparatively ready solubility (even at simple boiling temperature) of the glass of the culture vessels, the consequence being that a little alkali finds its way into the nutrient solution from the glass during the sterilising process. In order to eliminate this source of error several workers have already recommended the use of metallic culture vessels for the purpose in question, but soon had to abandon same on account of the toxic action and consequent retardation of development produced on the sowing. This was observed, in the case of silver, by RAULIN (III.), whose similar observation in the case of tin was confirmed by W. BENECKE (III.). This last-named worker also excluded aluminium from the list of suitable metals, for the reason that it sustained corrosion and therefore caused an alteration of the nutrient solution, although TH. BOKORNY (I.) employed it, apparently

with good results, in his experiments on the nutrition of *algæ*. Finally platinum, which, according to Bokorny, has a poisonous effect on the green thallophytes in question, was found by Benecke to be innocuous in the case of *Aspergillus niger*; it is, however, too expensive to use for large series of experiments. The difficulties encountered in purifying the nutrient salts, in order that the experiments conducted therewith may be perfectly reliable, can best be appreciated by the aid of the following data, for which we are indebted to W. Benecke (II.) and E. Günther (I.). The figures relate to the minimum quantity of KCl which will enable the development of the sowing to proceed when added to 100 c.c. of a nutrient solution previously free from potash. *Aspergillus niger* is sensitive to 0.02 mg., *Rhizopus nigricans* to 0.01 mg., *Mucor corymbifer* to 0.02 mg., and *Botrytis cinerea* to 0.01 mg. of KCl. The essential requirements of the fungi in respect of potash are therefore very moderate; and, in fact, if the necessary quantity be exceeded, to the extent of several units per cent., the growth may be injured. The maximum amount of potash salts which *Rhizopus nigricans* will stand, and still continue not only to grow but also to fructify, has been determined by E. Günther (I.) as follows: KCl, about 7.5 per cent.; KNO_3, about 7 per cent.; whilst in the case of K_2SO_4 the organism will still bear up to 10 per cent. (concentrated solution) very well.

In opposition to the concordant results obtained by earlier workers, Carl Wehmer (IV.) assumed that **sodium** is able to replace potassium as a nutrient material for fungi. This view was, however, disproved by the experiments of W. Benecke (III.) on *Aspergillus niger*, an undescribed species of *Penicillium*, *Mucor stolonifer*, *Botrytis cinerea*, and a pure-culture wine yeast from Winningen; these results being also strengthened by E. Günther's (I.) culture experiments with *Mucor corymbifer*, *Rhizopus nigricans*, and *Botrytis cinerea*. The rule therefore still holds good that sodium is of no appreciable utility as regards the nutrition of fungi, and can be entirely dispensed with. The maximum quantities of the salts of this metal that can be present in the nutrient solution without injury have been determined by E. Günther, in the case of *Rhizopus nigricans*, as follows: NaCl, 12 per cent.; $Na_2SO_4 + 10$ aq., 26 per cent.; and $NaNO_3$, 6 per cent.

With regard to **lithium**, W. Benecke (III.) has shown—in refutation of the contrary assumption by Nägeli—that this metal is not a foodstuff for fungi, although a strong stimulant. When lithium salts were present in the nutrient solution it was found that the conidia of *Aspergillus niger* did not germinate, and that no conidia were formed in the case of an unspecified species of *Penicillium*. The extent to which the various species are sensitive to the action of this metal must fluctuate considerably, since, whilst E. Günther (I.) found 0.05 per cent. to be the largest addition of lithium nitrate that *Rhizopus nigricans* could stand and still continue to thrive, H. M. Richards (I.) was able to observe that *Aspergillus niger* gave a crop of double the usual size when the nutrient solution of saccharose and mineral salts was treated with an addition of 0.3 to 0.5 per cent. of lithium chloride.

§ 229.—Metals of the Alkaline Earths.

According to Nägeli (IV.) **magnesium** is non-essential for the development of fungi when the latter have at disposal one of the three alkaline earth metals: **strontium**, **barium**, or **calcium**. The assumption that fungi can grow without magnesium has, however, been disproved by Winogradsky (XI.), who showed that the latter is indispensable for the development of *Mycoderma vini*. The same results were obtained by Adolf Mayer in culture experiments with beer yeast; by H. Molisch (II.) and W. Benecke (I.) with *Penicillium glaucum*

and *Aspergillus niger;* and by E. Günther (I.) with *Mucor corymbifer*, *Rhizopus nigricans*, and *Botrytis cinerea*. How sensitive and responsive the fungi are to a small addition of magnesium is evident from the observation recorded by Benecke (III.) as to the considerable difference in development exhibited by two, otherwise equal, specimens, the one grown without magnesium and the other in a medium containing 0.0025 mg. of crystalline magnesium sulphate per 25 c.c. Similarly, E. Günther (I.) ascertained, in the case of magnesium sulphate, ($MgSO_4 + 7$ aq.), that a minimum addition of 0.005 mg. was necessary to induce a sowing of *Rhizopus nigricans* to grow at all.

In refutation of an earlier assumption by Sestini, it has been shown by H. Molisch (II.) and W. Benecke (I.), and afterwards confirmed by E. Günther (I.), that not only calcium, barium, and strontium, but also the closely allied metals **beryllium**, **zinc**, and **cadmium** are unsuitable for replacing magnesium; and that, in fact, they behave as poisons when added in slightly larger amount to the nutrient medium. An addition of 0.02 *per mil* of cadmium sulphate or cadmium chloride is sufficient for *Aspergillus* and *Penicillium;* whilst for *Rhizopus nigricans* 0.001 *per mil* is enough. According to Günther, an addition of 0.2 per cent. of beryllium chloride is necessary to restrict the development of the last-named *Phycomyces*.

With regard to zinc, it was observed by J. Raulin (I. and III.) before 1870, in his experiments with *Aspergillus niger*, that the mycelial development of this fungus could be considerably facilitated by a small addition of zinc sulphate to the nutrient medium. The conclusion drawn therefrom that, in contradistinction to the earlier discoveries of the same worker, zinc is indispensable to the structure of the fungus in question was, however, unable to stand subsequent investigation. Both in this fungus and in the case of *Penicillium glaucum* and *Botrytis cinerea* it was found by W. Pfeffer (II.), H. M. Richards (I.), and Ono (I.), that the action of zinc is stimulative (§ 81) in the sense of H. Schulz's law (I.). Even an addition of 0.0005 per cent. of zinc sulphate to a nutrient solution of, *e.g.*, saccharose and mineral salts, resulted in a considerable increase in cropping. This attained double the yield (furnished in the absence of zinc) when the addition reached the optimum amount of about 0.003 per cent. of zinc sulphate; but, on raising the addition to 0.05 per cent., a poisonous action was observed. *Rhizopus nigricans* seems to be still more sensitive, since, according to E. Günther (I.), it will not stand more than 0.01 gram of zinc sulphate in 100 c.c. of nutrient solution. A noteworthy observation made by several workers is that this stimulation is really a kind of fattening process, the stimulative influence being confined to the development of the mycelium, that is, to the vegetative portion of the thallus; whilst the production of conidia, or organs of fructification, is retarded, and even entirely suppressed. Supported by other experimental results (relating to copper as well as zinc), Andr. Richter (I.) has pointed out that, in such a state of dilution, the salts (*e.g.*, zinc sulphate) are no longer capable of acting as such, but—in accordance with the theory of dissociation—are more or less separated into their components, the ions Zn and SO_4, which are therefore the real stimulants. Moreover, because the extent of the dissociation is also dependent upon the nature of the solvent—in this case the nutrient solution—the action exerted by an addition of such saline stimulants is also determined thereby. The present is a suitable occasion for referring to the labours of Th. Paul and B. Krönig (I. and II.), Scheurlen and Spiro (I.), and others, to whom we are indebted—since the publication of vol. i.—for the application of the dissociation theory to the study of the action of poisons on micro-organisms (§§ 79 and 81), and for the useful conclusions that may be drawn therefrom with regard to practical disinfection.

In connection with magnesium, indirect reference has already been made to

calcium, inasmuch as Nägeli's assumption that this metal could replace magnesium was stated to have been refuted. There still remains, however, the less important question whether calcium is at all essential to the structure of fungi. On this point Winogradsky, in a previously cited research, found *Mycoderma vini* still capable of thriving in a nutrient medium free from calcium (and also from barium and strontium). The same results were obtained by H. Molisch (II.) and W. Benecke (II.) in their experiments with *Aspergillus niger;* so we may well assume that calcium is not essential to the growth of fungi. Until a short time ago it was considered that this peculiarity afforded a thorough means of distinction between the fungi and green plants. However, it has recently been found by H. Molisch (IV.) that certain (but not all!) of the algæ will thrive in the absence of calcium, *e.g.*, *Stichococcus bacillaris*, Nägeli, *Ulothrix subtilis* Kützing, but not *Vaucheria* or *Spirogyra*. For the future, therefore, the axiom must be changed, and calcium regarded as indispensable for the higher green plants, but not essential to the fungi and to certain algæ.

So far as barium and strontium are concerned, it has been placed beyond doubt that these two metals are not only useless, but also injurious, and act as poisons toward the fungi. Thus, for example, in the experiments of E. Günther, the development of sowings of *Rhizopus nigricans* ceased in presence of 1.0 per cent. of barium nitrate, or 1.5 per cent. of strontium nitrate, in the nutrient solution. Even calcium, it may be remarked in passing, is capable of acting injuriously when present in larger quantities, the last-named worker having found the limit of safety to be 4 per cent. of calcium nitrate in the case of the same *Phycomyces*.

§ 230.—Elements of the Iron Group.

On the fact that **iron** had been shown indispensable to the formation of chlorophyll, and therefore essential to the assimilative power of green plants, was based the opinion, long unanimously current among mycologists—*e.g.*, Adolf Mayer (I.) in connection with yeast, and C. Nägeli—that the fungi, being destitute of chlorophyll, do not require iron. This assumption was first put to the test by J. Raulin (I.) in 1870, the results showing that, in presence of iron, cultures of *Aspergillus niger* throve more vigorously in solutions of nutrient salts containing iron, the weight of the crop being about double that obtained in cultures without iron. Presuming the experiments to have been properly conducted, this result would justify the assumption that iron, though very beneficial to the growth of the fungus in question, is not indispensable. Raulin, however, suspected that his "iron-free" cultures did not deserve their name, but contained a small proportion of this metal as an inseparable impurity in the other salts. On these grounds, though without proof, Raulin concluded that iron is indispensable for the development of *Aspergillus niger*. The contrary opinion, viz., that iron is non-essential to fungi, was expressed by Cugini (I.) in connection with a pileated fungus, and by A. Schulz (I.) for *Mycoderma cerevisiæ;* though, as neither of them worked with solutions that could be guaranteed free from iron, the question still remained undecided. Hans Molisch (I. and II.) attempted to settle it, without, however, succeeding in producing a perfectly iron-free culture. Nevertheless, his results tend to prove the indispensable character of the element in question, inasmuch as, in cultures as free as possible from iron, the spores of *Aspergillus niger* did not develop beyond the formation of a sickly mycelium, whereas the specimens treated with an addition of iron not only exhibited luxuriant growth, but also produced an abundance of spores. Similar results were obtained with sowings of pressed yeast cells, spores of *Mucor*

racemosus, and a species of *Penicillium*. Bearing in mind the observation (communicated by Molisch) that a ferruginous ash is furnished even by cultures grown in a medium from which iron has been, as far as possible, eliminated, and therefore that even imponderable amounts of iron are greedily absorbed, one is constrained to share the conclusion formed by Molisch, that iron is very probably indispensable to the development of fungi. The objection urged by C. Wehmer is based on observations that cannot be regarded as perfectly reliable. The hypothesis was raised by H. Molisch (I.), and shortly afterwards by A. B. Macallum (II.), that iron occurs, in plants generally and in fungi particularly, in the form of organic compounds, and is therefore undetectable by the ordinary reactions. Macallum (I.) then explained that the chromatin in the nuclear structure (§ 252) of the cell is the chief seat of these organic compounds of iron. The instances cited in support of this view, however, have been strongly criticised, since they were based on experimental methods the unreliability of which has been demonstrated by Arthur Meyer, Carl Müller (I.), and G. Gilson (I.), and also partly admitted by Molisch (III.) himself. Nevertheless, as may be concluded from later observations, the assumption itself seems appropriate. The first of these observations was made in 1877 by Lubavin, who detected the occurrence of iron in the molecule of the paranuclein prepared from milk casein. Further reports in this connection were made by A. Ascoli (I.), who succeeded in ascertaining the important fact that the plasmic acid (§ 252) isolated from the nuclein of yeast contains about 1 per cent. of (masked) iron, which is probably attached direct to the phosphorus atom. Attempts have already been made to utilise this fact in pharmacy and medicine; and since 1900 there has been recovered from yeast grown on a highly ferruginous nutrient medium a fission product of nuclein, to which the name **ferratogen** has been given, and which contains about 1 per cent. of iron in organic combination and readily absorbed in the intestines. According to G. Marpmann (II.), the iron in fungi (and especially in *Penicillium*) is usually in the ferrous state, and only exceptionally present in a higher stage of oxidation; this has been demonstrated by treatment with hydrochloric solutions of potassium ferro- and ferri-cyanide. An exception is afforded—at least according to R. Kusserow (II.)—by yeast (pressed yeast in particular), the stability of which is said to be influenced by its abundant content of ferric phosphate. In addition to its part as an indispensable foodstuff, iron also seems to act as a stimulant: according to the indications afforded by comparative experiments undertaken by H. M. Richards (I.).

The metals allied to iron, namely, **nickel**, **cobalt**, and **manganese**, have been tested on *Aspergillus niger* by Molisch (II.) with regard to their capability of replacing the first-named, but were found unsuitable. This does not, however, imply that the fungi entirely reject these elements. On the contrary, it has been shown by W. Pfeffer (II.) and H. M. Richards (I.) in cultures of *Aspergillus niger* and *Penicillium glaucum*, that cobalt and nickel, in the condition of sulphates, can exert a similar stimulative action to that already ascribed to zinc (§ 229). The optimum quantity of the salt of the first-named metal was found to be 0.002 per cent., which gave a crop almost double that from the check experiment without cobalt. In the case of nickel sulphate the corresponding quantity is 0.033 per cent. (consequently much greater), and this increases the crop two and a half to three fold.

As long ago as 1884, **manganese** was detected by J. Schlossberger and O. Döpping (I.) in various mushrooms—*e.g.*, *Agaricus deliciosus*, *Ag. arvensis*, and *Amanita muscaria*—and subsequently by Bissinger (I.) and others in *Lactarius piperatus*. Both physically and chemically this element seems to play a very noteworthy part. From what has already been stated in § 215 of vol. i.,

the reader is aware that many fungi contain oxidising enzymes allied to the laccase in the sap of the lac tree. Now, in the ash of the latter, Gabriel Bertrand (III.) has discovered up to 2.5 per cent. of manganese. Subsequent experiments have led this worker to conclude—though this has not yet been confirmed—that manganese is the real active agent in the oxidases, by reason of the convertibility of its protoxide, this being readily oxidised to peroxide, which in turn as readily parts with oxygen and is reduced to the protoxide. Consequently, in Bertrand's (VI.) opinion, the organic constituents of the oxidases merely play the part of carriers of manganese. For the purpose in view, this latter cannot be replaced by any allied or other metal.

According to the results obtained by Richards (I.) in culture experiments with *Aspergillus niger*, **aluminium** is not only non-essential as a foodstuff, but has no appreciable action as a stimulant.

§ 231.—Sulphur, Selenium, Silicon, Phosphorus, Arsenic.

Strictly speaking, it has not yet been proved that **sulphur** is essential to the growth of fungi; that it is so having been concluded from the (still disputed) assumption that this element forms an important constituent of the albuminoids. The attempts hitherto made to carry out perfectly convincing experiments, in nutrient media, positively free from sulphur, have proved futile. Thus, neither Adolf Mayer nor E. Günther (I.) succeeded in fully eliminating this element from the saccharose used in the preparation of nutrient solutions, a few thousandths of a per cent. remaining in combination as an ineradicable impurity. According to the investigations of Nägeli, sulphates as well as sulphites and hyposulphites may serve as a source of sulphur; but ammonium thiocyanate and sulphurea are unsuitable. A careful confirmation of this report is the more desirable because Adolf Mayer, in his cultures of beer yeast, found sulphates unsuitable for this purpose.

Selenium appears to be incapable of replacing its near ally, sulphur, as a nutrient material for fungi. At any rate, the experiments of E. Günther (I.) with *Rhizopus nigricans* have shown that an addition of even 0.0005 per cent. of sodium selenate will suffice to prevent the germination of spores of this fungus in a nutrient solution of glycerin and mineral salts.

Silicon also, according to J. Raulin (III.), must be included in the list of foodstuffs essential to fungi, though no support to this view is afforded by the later cultivation experiments conducted on this point by H. M. Richards (I.). Nevertheless—in view of the observation (unfortunately not followed up) of E. Winterstein (I.) that the ash of his so-called fungocellulose (§ 225) consisted almost exclusively of silica—it may be regarded as not impossible that silica (as is undoubtedly the case in the higher plants), while not *essential* to the structure of fungi, is very useful for strengthening their membranes. The present is a suitable opportunity for mentioning a nutrient solution (*Liquide Raulin*) still used in French laboratories, for both moulds and fission fungi. It was compounded by Raulin (I. and III.) on the basis of his observations, which we now know to have been somewhat imperfect, and is composed of:

	Grams.		Grams.
Water	1500.0	Magnesium carbonate	0.40
Saccharose	70.0	Potassium carbonate	0.60
Ammonium nitrate	4.0	Tartaric acid	4.00
Ammonium phosphate	0.6	Ferric sulphate, zinc sulphate, potassium sulphate, each	0.07
Ammonium sulphate	0.4		

Next to potash, **phosphoric acid** is the most important ash constituent in fungi. Existing analyses place the figures at between 15 and 60 per cent.;

though it should be mentioned that not all the reports on this point are of equal value, some of them relating to cases where insufficient regard was paid to the volatility of phosphoric acid in presence of carbon, during the preparation of the ash. The consensus of existing experiments favours the assumption that phosphorus is essential to the structure of fungi; and, moreover, it is known that this element forms an important constituent of the nucleins (§ 252). The eagerness with which this foodstuff is absorbed from the medium can be gathered from an instance recorded by J. Schlossberger and O. Döpping (I.), wherein a pileated fungus, *Dædalea quercina*—of the *Polyporea* group, and allied to the dry-rot fungus—was found to have completely extracted the phosphoric acid from decayed oak wood. With a view to ascertaining the local distribution of phosphoric acid among the individual constituents of the cell contents, L. Lilienfeld and A. Monti (I.) have described a process, which consists in first immersing the preparation in a nitric solution of ammonium molybdate, whereby a yellow precipitate of ammonium phosphomolybdate is produced in such parts of the cell where phosphoric acid, capable of reacting, is present. Then, after washing out the preparation, it is exposed to the action of a reducing agent (such as a 20 per cent. solution of pyrogallol); this reduces the molybdic acid to lower stages of oxidation, which, by their brown or blue colour, reveal the locality and distribution of the phosphoric acid in the cell. In this manner the hyphæ of *Botrytis cinerea*, the spores of the same fungus, and the cells of *Mucor* and *Saccharomycetes*, have been tested for phosphorus by S. Pollacci (I.). According to its authors, the method is capable of detecting not merely the phosphoric acid in combination as inorganic orthophosphates, but also, in many cases, that present in organic combination, especially in the nucleins. It has, however, been found by L. Heine (I. and II.) that the same colour reaction is furnished by various substances devoid of phosphorus—many albuminoids in particular—so that it is impossible to obtain by this method any reliable differentiation, especially between the nuclein-like and the albuminoid constituents of the cell.

The question how far phosphorus can be replaced as a foodstuff by its ally, **arsenic**, after having been answered in the negative by J. Stoklasa for the higher phanerogams, and by H. Molisch (IV.) for different algæ, was examined by E. Günther (I.) in connection with *Rhizopus nigricans*, the same result being obtained. This notwithstanding, compounds of arsenic are attacked and assimilated by many fungi, provided they are present in insufficient quantity to exert any poisonous action. B. Gosio (II.) was the first to show that *Penicillium glaucum*, *Aspergillus glaucus*, *Asp. virens*, *Mucor mucedo*, *M. racemosus*, *Cephalothecium roseum*, and others, in suitable nutrient media will convert arsenious acid into volatile compounds having an odour of garlic, and with such a degree of certainty that an addition of 0.02 mg. of Na_2AsO_3 can be detected in 10 c.c. of milk by this means. This new fact is of practical interest to official chemists and hygienists, in connection with the toxic phenomena produced by wall-papers, &c., containing arsenic. The chief literature on this subject has been collected by R. Abel and P. Buttenberg (I.). Attempts have been made to explain these phenomena by assuming that the printed arsenical pigments come off in the form of dust; and this view was recently accepted by O. Emmerling (I.), since neither he, nor G. Marpmann (V.) since, have succeeded in liberating arseniuretted hydrogen from cultures of the aforesaid fungi (or of *Mucor corymbifer* and *Aspergillus fumigatus*) on arseniferous nutrient media. On the other hand, the probability that volatile organic compounds of arsenic are formed has been shown by Gosio (IV.); and one such has been made known by P. Biginelli (I.) as diethylarsine, $As(C_2H_5)_2H$, which is a near ally of the extremely poisonous substance cacodyl and emits an odour of garlic. The advantage accruing to the analyst from Gosio's observation is the possibility of

replacing the Marsh test for arsenic by a shorter, microbiological method, wherein the substance under examination can be tested without the necessity for a tedious preparation (destructive of the organic matter!). The method as recommended by Gosio is performed in the following manner: A cut is made in a clean piece of raw potato, and after inserting therein a little of the substance (*e.g.*, aniline dyestuff) to be tested for arsenic, the potato is placed in a Roux test-glass (potato glass) and sterilised for fifteen minutes under a pressure of one atmosphere. Abel and Buttenberg (I.) recommend the employment of a pap made from the crumb of white or brown bread and contained in an Erlenmeyer flask; and this has been found useful in the present author's laboratory. When cooled, the substratum is inoculated with an organism known to be suitable for the purpose in view, and the whole is then left to stand for a day at about 37° C. If the substance contain arsenic, the contents of the tube or flask will, at the expiration of that period, emit a strong and persistent odour of garlic. Gosio cites, as the most energetic assistant in this connection, the (presumably new) *Penicillium brevicaule* discovered by him on arsenical wall-paper. In order to be sure in the first experiment, the liberated vapours may be passed into an oxidising liquid, *e.g.*, permanganate solution, and examined by the Marsh test. By the aid of this microbiological method Fr. Abba (I.) succeeded, in a case under judicial investigation, in proving the presence of arsenic in a sample of Indian meal; then in the urine of a patient treated with arsenic; and also in illuminating gas from the Turin mains, the arsenical constituents being collected by passing the gas through caustic potash. In the same manner, several hundred samples of pelts were tested for arsenic (§ 157) by Gosio (V.), and also later on by Abba (II.). In proof of the delicacy of the method, the last-named worker states that, whilst, with the Marsh apparatus, no arsenic could be detected in a piece of hide measuring 5 sq. cm., an unequivocally affirmative answer was obtained by the biological method from a piece one-fiftieth the size, *i.e.*, only 10 sq. mm. This method has also been employed, and its delicacy appreciated, by G. Mopurgo and Alb. Brunner (I.) for the examination of colouring-matters used in the provision industry. Samples containing not more than 0.2 mg. of arsenious acid per 10 grams gave merely a very slight, doubtful mirror in the Marsh apparatus; but when treated in the manner prescribed by Gosio they disengaged a strong odour of garlic within a few hours. A still more favourable opinion is expressed by W. Scholz (I.), R. Abel and P. Buttenberg (I.), Br. Galli-Valerio and C. Strzyzowski (I.), who found the limits of delicacy of the process correspond to 0.02 to 0.05 mg., and 0.001 to 0.01 mg. of arsenic respectively. The reaction was not given by other metallic poisons, such as compounds of antimony, lead, and bismuth in particular. Out of more than forty different species of mould fungi that have up to the present been examined with regard to their suitability for this method, *Penicillium brevicaule* has proved *facile princeps*. On the other hand, *Aspergillus flavus*—which, according to R. Schmidt (I.), has a very powerful reducing action—*A. niger*, *A. subfuscus*, *A. fumigatus*, *Penicillium glaucum*, *Mucor mucedo*, and others, have been found unsuitable, the odour of garlic being either entirely absent or else masked by the fusty smell of the mould fungi.

In view of the details given in the foregoing, it may be stated that, according to the present state of our knowledge, nine elements are indispensable for the structure and complete development of the *Eumycetes*, namely—

Carbon, hydrogen, oxygen,
Nitrogen, sulphur, phosphorus,
Potassium (or rubidium), magnesium, and iron.

CHAPTER XLII.

STIMULATIVE INFLUENCES—GENERAL REMARKS ON THE ENZYMES OF EUMYCETES.

§ 232.—Influence of Light on the Development of the Eumycetes.

According to an old colloquial expression, the fungi are children of darkness. This statement, however, is only appropriate in certain instances, more particularly in the subterranean species, *e.g.*, the truffle, and is by no means capable of general application. The first to protest against this generalisation was E. M. Fries, who showed, in 1825, that light is indispensable to the normal development of certain fungi. Following his lead, a large number of workers have since investigated the connection between light and fungoid life; but only a few of the results obtained can now be briefly recapitulated, namely, those concerned with the fungi of interest to readers of the present work. We will also leave out of consideration the older reports dealing with the malformed growths—due to defective illumination—of fungi in mines, where they drag out a miserable existence; and also the (barren) mycelial masses found in mine shafts and headings, and described, in the older literature, under the collective name of *Byssus*. Information on this question will be found in a work by Elfving (I.), to which we shall have occasion to refer later on.

The first phenomenon we shall now consider is one that will become apparent on even merely superficial observation, namely, **phototropism**, or the influence exerted by light on the direction of growth. At the outset research was confined to the narrower field of the form of illumination most common under natural conditions, viz., by the sun's rays (**heliotropism**); and, according as this influence proved stimulative, retardative, or inert, the fungi affected thereby were classed as positively heliotropic, negatively heliotropic, or aheliotropic. An example of each of the two latter possibilities was furnished by J. Schmitz (I.) in 1843 and by Kraus (I.) in 1876, the latter of whom found *Rhizopus nigricans* (*Mucor stolonifer*) presumably aheliotropic. Schmitz observed that the mycelial threads at that time classed independently as *Rhizomorpha*, but subsequently assigned to the cycle of development of *Agaricus melleus*, turned away from the light, though Brefeld (III.) was unable to confirm this behaviour. E. Chr. Hansen (XXII.) made us acquainted, in 1897, with three new examples of negative heliotropism, in species of the families *Coprinus* and *Agaricus*. With regard to all the remaining fungi examined for their sensitiveness to light and found to be exclusively positive in their heliotropism—such, for example, as the conidiophores of *Peziza Fuckeliana* examined by G. Winter (I.), the sporangial hyphæ of *Mucor mucedo*, *Phycomyces nitens*, and a species of *Pilobolus*, examined by Kraus (I.) and Vines (I.), and the stalk of *Coprinus lagopus*, examined by Brefeld (III.)—an advance was then made by separately examining the influence of the different colours of the spectrum on growth. No uniform results, however, were obtained; for, whereas Fischer von Waldheim (I.) found that *Pilobolus cristallinus* was only heliotropic under blue light, both its congener, *Pilobolus microsporus*, and *Mucor mucedo* are also sensitive to yellow light, according to Brefeld (IV.) and Regel (I.). The further researches of Wiesner (I.), in contradiction to those of

Fischer von Waldheim, show that *Pilobolus cristallinus* and *Coprinus niveus* still continue to turn heliotropically, even in the ultra-red rays. We are indebted to Friedr. Oltmanns (I.) for the settlement of this discrepancy, and also for raising the consideration of this phenomenon to a higher plane than before. His researches were performed on *Phycomyces nitens;* that is to say, the very fungus that had hitherto been regarded as decidedly positive in its heliotropism, and the one chiefly used in lecture demonstrations on account of the unusual sensitiveness of its long sporangial hyphæ to light. By using a very powerful electric arc light (up to 5300 Hefner units), Oltmanns found that the fungus in question behaved positively phototropic under weak illumination, but negatively so under a powerful light, whilst at an intermediate stage of illumination it remained aphototropic. The universal law of stimulants thus applies also to the phototropy of fungi, the sign—to speak mathematically—of the stimulative effect being determined by the strength of the influence. However, the degree of stimulation necessary to the production of a given effect is also dependent on the actual condition of vitality of the individual under examination, age, in particular, being an important factor. Thus, in the case of *Phycomyces nitens*, a given degree of illumination causes attraction in the young sporangial hyphæ (with just grey sporangia), whereas in the older ones (with already blackened sporangia) it induces repulsion. The applicability, to the phototropism of fungi, of Weber's law (§ 233) of the ratio between the degree of stimulation and the effect, has been demonstrated by J. Massart (III.) in the case of *Phycomyces nitens.* The existence of after-effects of stimulation was first remarked, in this connection, by Wiesner (I.).

Although the influence of light on all the other vital manifestations of fungi has been the subject of numerous observations and experiments, no uniform results have been secured, nor has the same elevated point of view been attained as is the case—thanks to Oltmanns—with regard to its influence on the direction of growth. In dealing with this next question, we will follow the natural course of development, and begin with the **influence of light on the germination of the spore.** Now, according to H. Hermann (II.), or rather E. Löw (III.), germination is not affected in the case of the conidia of *Penicillium glaucum*, *Tricothecium roseum*, and *Fusarium heterosporium*, or the endospores of *Rhizopus nigricans* (*Mucor stolonifer*). Contrary results were afterwards obtained by von Wettstein (I.), who found germination retarded by light in the case of spores of *Rhodomyces Kochii;* and by F. Elfving (I.), who proved that intense sunlight entirely prevents germination in the conidia of *Aspergillus glaucus.*

The reports of experimenters also differ with regard to the **influence of light on the vegetative development**—increase in the size of the cells, and the power of growth. Thus, whereas, according to a report by J. Schmitz (I.), *Sphæria carpophila* grows more strongly in the dark than in daylight, *Peziza Fuckeliana* —according to G. Winter (I.)—ceases to grow in the dark, and perishes entirely if the exclusion of light be prolonged. Van Tieghem (IX.) and Gaillard (I.) found illumination exercise a favourable influence on the development of *Penicillium* and certain yeasts respectively. Kraus (I.), on the other hand, found the perithecial hyphæ of *Clariceps microcephala* attain their greatest length in the dark, rather than in red, yellow, or blue light. Brefeld also made the same observation with regard to the stalks of certain species of *Coprinus.* G. H. Vines (I.) found the growth of the sporangial hyphæ of *Phycomyces nitens* prejudicially affected by light, and traced this action to the influence of the blue rays.

The first to investigate the **influence of light on cell fission,** and therefore on **cell reproduction,** was L. Kny (I.), in the case of pressed yeast. He failed to discover any difference in the rate of reproduction in the dark and under

moderate illumination by gaslight. This is, however, altered in the case of strong insolation, as will be referred to later on.

Also **in respect of fructification** the individual species of fungi seem to differ with regard to the strength of illumination necessary to the production of a given result; such at least is the conclusion furnished by comparing the reports of various observations conducted on this point, the original probability, based on considerations of a general physiological character, being heightened by the determinations of Oltmanns. According to H. Hoffmann (II.), E. Löw (III.), and A. Lendner (I.), the formation of sporangia or conidia in *Rhizopus nigricans*, *Thamnidium elegans*, and *Mucor mucedo*, or *Penicillium glaucum* and *Trichothecium roseum* respectively, proceeds just as well by daylight as in the dark. C. Werner (I.) also failed to detect any influence of light on the formation of conidia in two of the higher *Ascomycetes*. In the case of *Botrytis cinerea*—according to Rindfleisch (I.)—this formation occurs solely by night; and in this instance, as was determined by L. Klein (III.), the retardation is attributable mainly to the blue-violet rays of the spectrum. The converse has been observed in the case of *Rhizopus nigricans*, which, according to A. Lendner, puts forth its sporangia two days later in the dark, or in red and yellow light, than in white, blue, or violet light. In the dark, *Mucor racemosus* produces merely barren sporangia; whilst, in the case of the *Thamnidium aurantiacum* described by Rochard (I.), this fructification is said to proceed most favourably in twilight, and to be prejudiced and retarded by strong light as well as by an absence of illumination. This report, however, has been contradicted both by Payen (I.) and Poggiale (I.). A similar observation to that of Rochard was made by A. Lendner with regard to the formation of conidia in *Aspergillus luteus*, *Asp. niger*, and *Botrytis* (*cinerea ?*). Elfving (I.) states that the formation of perithecia in *Aspergillus glaucus* is entirely or to a large extent suppressed by light; and the same applies to the formation of the pileus in certain members of the genus *Coprinus*—congeners of the mushroom—observed by Brefeld (III.).

Nevertheless, the question of the influence of light on the fructification of any given fungus cannot be answered off-hand. On the contrary, it has been established beyond doubt that the nature of the action exerted by light depends on the other conditions of vitality, Brefeld (III.) having shown, in the case of *Coprinus stercorarius*, that the formation of the pileus ceases in the dark when the temperature remains below 15° C. This discovery that light rays can be replaced by heat rays becomes of great interest when it is borne in mind that—as was determined by Brefeld—the only light rays having any influence on that development are the blue-violet ones, and not the less refractive (*e.g.*, the yellow) rays. A determinative influence is also exercised by the composition of the nutrient substratum. Thus, A. Lendner made the observation—which deserves following up—that sporangiation in *Mucor flavidus* occurs in white light, but ceases in yellow or red light or in the dark, when the organism is grown in Raulin's nutrient solution (§ 231); but that the converse is the case when van Tieghem's nutrient solution is used.

Grown on solid media this fungus seems to develop sporangia under any kind of illumination. A fact of not less importance than the **capacity of heat rays to replace light rays** is that demonstrated by Brefeld (IV.), in the case of *Pilobolus microsporus*, a fungus of the *Mucoracea* family (§ 235), namely, the **after-effect of illumination**. When kept in the dark from the beginning, the mycelium of this fungus remains entirely and permanently barren; but if exposed to the light for a couple of hours, and then placed in the dark before any signs of fructification appear, the sporangia will develop, though ordinarily they only do so in the light.

Still more scanty is our knowledge of the **influence of light on the internal life**, *i.e.*, the chemico-physiological capacity, of the *Eumycetes*. In fact it is

almost entirely confined to a series of observations made on **respiration** (§ 238), *i.e.*, **the exhalation of carbon dioxide**. The first experiments were conducted by Wilson (I.), who failed to discover any influence due to light; and also by Bonnier and Mangin (I.), and Puriewitsch (II.), who experimented with various pileated fungi (*inter alia*, *Agaricus campestris*) and *Phycomyces nitens*, and in all cases found respiration hindered by diffused daylight, the strongest effect being produced by the least refrangible rays. These experiments, however, were of a merely preliminary character, no regard having been paid to the circumstance that the exhaled carbon dioxide may originate from *two* distinct vital processes; viz., either from the conversion of matter in the course of building up the cell, or from the process of combustion within the full-grown cell for the purpose of replacing dissipated internal energy. Duclaux (X.) was the first to point out to mycologists this difference, which was already well known in animal physiology. And, as a matter of fact, the subsequent work of Elfving (I.)—on *Mucor racemosus*, *Aspergillus niger*, *A. flavescens*, and *Penicillium glaucum*—led to the discovery that this differentiation of the two sources of carbon dioxide is also applicable in the case of fungi. In older cultures, wherein the foodstuff is chiefly consumed in order to maintain the vital energy, no apparent influence is exercised by light on the volume of exhaled carbon dioxide. On the other hand, a retarding effect is produced by light in younger cultures, *i.e.*, those in which the reproduction and growth of the cells is proceeding briskly. All the above-mentioned workers regarded the total amount of carbon dioxide, liberated by the cultures under observation, as a measure of the respiration; but R. Kolkwitz (I.) took into consideration, as a by no means negligible source of error in prolonged experiments, the gradual decomposition sustained by oxalic acid under the influence of light (§ 21), since this acid is of frequent occurrence among the metabolic products of fungi. On excluding this source of inaccuracy, by examining the cultures under a brief illumination by a powerful electric arc, this worker ascertained that considerable acceleration of respiration is experienced in the case of *Oidium lactis*, *Aspergillus niger*, and one species each of *Mucor* and *Penicillium*.

The **action of sunlight** has been the object of a number of observations, which merit our attention the more in that they were chiefly made with yeasts, and partly relate to the biology of the fungoid flora of the vine. Of the other fungi, *Aspergillus glaucus* was examined by Elfving (I.) in this connection, whereby it appears that the conidia when ripe will stand insolation for several weeks in succession at the latitude of Helsingfors, without injury, though they are killed in a few days when in a young immature condition. Far inferior powers of resistance were presented by the yeasts subjected to insolation in the south of France by V. Martinand (II.), whose results were afterwards confirmed by G. Tolomei (VII.). It was found that both sporogenic and sporeless cells in two races of the group *Saccharomyces ellipsoideus*, and also cells of *S. apiculatus*, perished after four hours' exposure to the sun's rays at an atmospheric temperature of 41° to 45° C. A similar result followed insolation for three days at 36° to 37° C., whereas other specimens of the same species remained alive when kept in the dark under otherwise equal conditions. This coincides with the results of the experiments made by W. Lohmann (I.) with *Saccharomyces Pastorianus I.*, Hansen, two species of *Torula*, two film-yeasts (*Mycoderma*), and a distillery yeast (Race II. of the Berlin Experimental Station), these organisms being killed in a few hours by insolation, as well as by illumination with an electric arc lamp of 8000 to 11,540 metric candle-power. The last-named culture yeast succumbed first, whereas the first of the wild yeasts proved the most resistant. The unfavourable conditions artificially produced by Martinand are experienced in practical viticulture by the yeast cells that make their habitat on the grapes

most fully exposed to the sun, *i.e.*, particularly those on the upper part of the vine-stocks. Consequently we should expect to find in that position a smaller number of living cells than on the grapes lower down. This observation of Martinand's also leads to the conclusion that the grapes from southern countries —which often give very poor fermentation—are less abundantly inhabited by yeast cells, and are chiefly infested by such races as are less sensitive to insolation. It is therefore probable, as a result of this factor of natural selection, that the wine yeasts of southern latitudes are physiologically different from those whose progenitors have lived for centuries under a cooler sky.

An assertion deserving closer investigation is that of Ward (VII.), to the effect that the colouring-matters of chromatic fungi afford protection against the injurious influence of light. Ward supports this view by the personal observation that the colourless spores of *Oidium lactis*, *Chalara mycoderma*, and *Saccharomyces pyriformis* were killed by insolation, whereas this was not the case with those of *Aspergillus glaucus*, *Penicillium crustaceum* (*P. glaucum*), *Mucor racemosus*, and *Botrytis cinerea*. It must not, however, be forgotten that the greater power of resistance offered by these spores may well be due to the considerable thickness of the membrane. Nevertheless, as the foregoing discoveries by Elfving have shown, all means of protection fail in presence of prolonged insolation.

With regard to the **influence of the Röntgen rays** on the vitality of the *Eumycetes*, an experiment was conducted on *Phycomyces nitens* by L. Errera (I.), but furnished no definite results. Further research on this point is therefore desirable.

§ 233.—Chemotropism—General Remarks on the Enzymes of Eumycetes.

Chemotaxis has already been explained (§ 41 of vol. i.) as the attraction or repulsion of motile micro-organisms by chemical stimulants. Motile cells, which are therefore capable of being similarly influenced, are also found among the *Eumycetes*, namely, the zoospores (§ 220) of the *Oomycetes* and *Chytridiaceæ*, *i.e.*, fungi that do not come within the scope of the present work. In the case of the other higher *Eumycetes*, the effects of such a stimulus are manifested by the affected individual either developing with particular strength towards the seat of the stimulus (inclining thereto) or turning in the opposite direction. These phenomena are termed respectively **positive** and **negative chemotropism**. The earliest statements and observations on this point were made by W. Pfeffer (IV.), then by J. Wortmann (VIII.), M. Ward (VI.), M. O. Reinhardt (I.), and others. More thorough investigations were conducted by M. Miyoshi (I.) with *Mucor mucedo*, *Phycomyces nitens*, *Rhizopus nigricans*, *Penicillium glaucum*, and *Aspergillus niger*, in various ways ; *inter alia*, by sowing the spores of these fungi on the surface of a finely perforated mica plate, the lower side of which was in contact with the solution of the substance under test. The following were found to act as powerful stimulants on these fungi: ammonium phosphate, among the inorganic substances; and asparagin, dextrin, and various sugars (saccharose and glucose) among organic compounds. Glycerin proved almost entirely inactive.

The minimum quantity of any stimulant capable of producing a chemotactic or chemotropic effect on fungi had already been termed the **marginal limit** (*Schwellenwerth* = literally, "threshold value") by W. Pfeffer. This value was ascertained by Miyoshi (I.) as 0.01 per cent. in the case of glucose acting on *Mucor mucedo* or *Rhizopus nigricans*. On gradually increasing the dose above

this minimum limit, a second limit is reached whereat the effect is one of repulsion, not attraction. Thus, in the case of Miyoshi's experiments, the integument of the spores of *Rhizopus nigricans* just began to be appreciably attracted by a 0.1 per cent. solution of saccharose, the effect then increasing with the dose, until, at about 15 per cent., diminution commenced; and repulsion could be detected at about 50 per cent. The maximum distance at which the influence of the stimulant could be detected was, in the case of the germ tubes of *Rhizopus nigricans* and *Phycomyces nitens*, about twelve to fifteen times the longitudinal diameter of the spores.

Two different explanations have been put forward as to the nature of the intimate causation of negative chemotropism. Massart (I.) assumes that **osmotic action** is really in question, and that consequently the repulsion of the fungus from the seat of the stimulus is a result of **plasmolysis**, due to the high concentration of the solution. On the other hand, Pfeffer (IV.) and Miyoshi found a series of substances which produce an effect of repulsion in all stages of concentration: *e.g.*, potassium chlorate, alcohol, all the acids so far examined, &c. Consequently both workers hold the opinion that this effect cannot be explained as the result of osmosis (like the case of the other substances), but is attributable to a specific power of repulsion.

The relation between the strength of the stimulus and the degree of effect produced was investigated by Pfeffer, and found to agree with the general law enunciated by Weber, viz., "The magnitude of the reaction increases in arithmetical progression as the magnitude of the stimulus increases geometrically." Of course the application of this law is here confined within certain limits, and is therefore excluded when *positive* chemotropism begins to turn into *negative* in consequence of a strongly increased concentration of the stimulant.

Further investigation is merited by the question of how far chemotropic action is concerned in the phenomenon known as **rheotropism**, the outward sign of which is the adoption by fungoid hyphæ of a definite position with respect to the direction of flow of the surrounding liquid. Jönsson (I.), to whom we are indebted for the first observation on this point, found that the mycelial hyphæ of *Botrytis* grow against the current, but those of *Mucor* and *Phycomyces* with the stream. The first-named fungus he termed positively rheotropic, the other two negatively so.

The tendency of fungoid hyphæ to grow towards a positively chemotropic stimulus is manifested not merely when the latter is freely accessible, but also when it is separated from the influenced fungus by a partition. In some species and under special conditions this tendency is expressed by the excretion of substances capable of attacking and perforating the said partitions. Among such substances may be mentioned carbon dioxide and oxalic acid, which come into action more especially when the fungus is in contact with a calcareous substratum, such as an egg-shell or a bone. On this point a number of experiments were conducted by K. Lind (I.) with *Aspergillus niger*, *Penicillium glaucum* and *Botrytis cinerea*.

Of the organic substances naturally forming such a partition wall, viz., either chitin or cellulose, together with allied carbohydrates, the first comes under consideration in all cases where the fungus endeavours to perforate the shell of an insect. In order to effect this it has need of an enzyme capable of dissolving chitin, on which point several reports have been made by Zopf (X.). However, even when the object infested with the parasitic fungus is a fungus itself, the collaboration of a similar enzyme is necessary, since chitin (§ 226) forms a principal constituent of the membrane of many members of this class. The faculty of excreting an enzyme capable of dissolving cellulose and allied carbohydrates becomes mainly apparent when higher plants are infested by a fungus.

The first notice of the occurrence of such an enzyme in *Eumycetes* was that of DE BARY (II.) in 1886, in the case of *Sclerotinia* (*Peziza*) *Libertiana;* and a similar one was recorded in 1888 by M. WARD (I.), in an allied species injurious to lilies. A year later, E. Kissling found the same faculty in *Sclerotinia Fuckeliana*, and this was confirmed by J. BEHRENS (IV.), M. MIYOSHI (II.), and M. NORDHAUSEN (I.). Subsequently, on the basis of his own experiments, M. O. REINHARDT (I.) came to the conclusion that the membrane-dissolving enzyme secreted by the first-named *Sclerotinia* is different from that of the second species. This is by no means surprising, in view of the great variety of carbohydrates (§ 227) taking part in the structure of the vegetable cell wall. The ability of *Botrytis cinerea* to secrete an enzyme capable of dissolving true cellulose has been demonstrated by an experiment carried out by J. BEHRENS (IX.) in a manner to which no objection can be raised. On the other hand, *Penicillium glaucum*, *P. luteum*, *Rhizopus nigricans*, and probably also *Monilia fructigena*, have been proved incapable, though, with the exception of the last-named species, they can attack the so-called central lamella (§ 119) and therefore secrete a pectin-dissolving enzyme. Another organism capable of dissolving true cellulose is the vine-root mould, examined by J. BEHRENS (XII.), and termed by him *Pseudo-Dematophora*. This is a non-pathogenic mould fungus occupying an unknown position in the botanical system; it grows generally on wood, and has a particular affinity for vine-wood, which it rots and destroys.

According to CZAPEK (II.), the lignified cell walls of higher plants consist of an ethereal combination of cellulose with a substance to which he gives the name **hadromal**. The dissociation of this compound and the liberation of cellulose are probably effected by an enzyme which this worker has discovered in certain fungi, *e.g.*, dry-rot (§ 80), and which he terms **hadromase**. Fungi that have invaded trees and timber find, in the bark and cambium layer of same, oftentimes large amounts of glucosides, such as salicin, populin, amygdalin, coniferin, &c. Thanks to the action of certain excreted enzymes, such as **emulsin**, they are able to utilise for their own nutrition the carbohydrate separable from these substances. A number of these parasites were examined in this respect, with affirmative results, by E. BOURQUELOT (III. and IV.). Further mention of *Eumycetes* enzymes capable of decomposing glucosides will be made, with reference to certain special examples, in a later section.

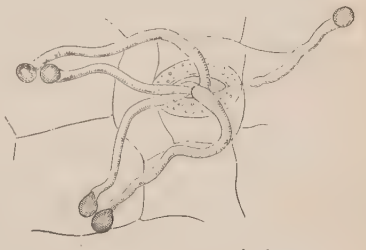


FIG. 110.—Rhizopus nigricans.

Hyphæ from five spores which, twenty-seven hours previously, were sown on the under surface of a leaf of *Tradescantia discolor* that had been injected with a 2 per cent. solution of ammonium chloride, making their way to the stoma, and passing therethrough into the interior of the leaf tissue. Magn. 100. (*After Miyoshi.*)

However useful to parasites may be the faculty of secreting an enzyme capable of dissolving the cell walls of the infested host, there are certain cases where the parasites can dispense with this faculty and still attain the end in view. This is effected by the purely mechanical pressure exerted by the apex of the hypha on the cell wall of the host, when the hypha itself has formed an appressorium (§ 237) and thus provided an abutment. In this manner it succeeds in perforating the cell wall. This faculty has been confirmed by M. MIYOSHI (II.) in the case of *Penicillium glaucum* and *Botrytis cinerea*, both of which proved capable of penetrating thin gold leaf quite free from holes. In this case chemical action was entirely precluded. When fungoid hyphæ obtain a footing on the epidermis of such parts of plants as exhibit stomata, they often prefer to obey the chemotropic attraction exercised by the cell contents, in a peculiar manner, by growing

towards the nearest stoma, passing, by this means, into the interior of the plant tissue, and then penetrating the cell membranes, which are far thinner than that of the epidermis. An example of this is shown in Fig. 110. The successful entrance of the hyphæ in this manner is not, however, invariably followed by the further development of the parasite, the sequel depending on the constitution of the cell contents. The latter, without prejudice to their property of attracting the invader, may be of such a character as to preclude its growth. Further information with regard to this biologically interesting and practically important question may be found in handbooks on plant diseases; and a few relevant remarks will also be made later on in connection with the rotting of fruit and "sweet-rot" in grapes.

In the absence of a more favourable opportunity, a few general remarks on the secretion of proteolytic enzymes (§ 170) by *Eumycetes* may be made here. The first record of a gelatin-dissolving *Eumycetes* enzyme was that made by Adolf Hansen (I.) in 1889, in cultures of *Mucor mucedo* and *Penicillium glaucum*. Two years later it was shown by A. Weidenbaum (I.) that *Oidium lactis* and *Oidium albicans* differ from one another, inasmuch as the latter does not cause the liquefaction of the gelatinous nutrient medium under any circumstances, whereas liquefaction is produced by the former organism when the medium has an acid reaction. According to Zopf (X.), *Hormodendron cladosporoides* is incapable of secreting such an enzyme. Subsequently, C. Wehmer (XII.) examined a number of mould fungi in this connection, and found that, when grown in a nutrient medium (beer wort, dextrose-nutrient-salt solution) containing 10 per cent. of gelatin, the following: *Aspergillus niger*, *A. oryzæ*, *A. candidus*, *A. minimus*, *A. Ostianus*, *A. novis*, *Penicillium glaucum*, *P. olivaceum*, *P. italicum*, *P. luteum*, *Botrytis cinerea*, and *Cephalothecium roseum* will liquefy about one-half the medium within ten days, and produce complete liquefaction in two to three weeks. The results were indefinite in the case of *Aspergillus glaucus*, *A. fumigatus*, and *A. varians*. According to later reports by the same worker, a slight liquefaction of the gelatin is also effected by *Mucor Rouxii* and *M. javanicus*. In the course of a comprehensive investigation on the occurrence and activity of a gelatin-dissolving enzyme in various members of the vegetable kingdom—including a number of fungi—Fermi and Buscaglioni (I.) obtained affirmative results with sundry edible fungi, as also with *Claviceps purpurea*, *Aspergillus flavus*, &c.

The occurrence of enzymes capable of dissolving casein was investigated by E. Bourquelot and H. Hérissey (I.), who found such a one present in about 20 out of 126 species of fungi examined, *e.g.*, in *Amanita muscaria*, *Boletus edulis*, &c. Both workers hold this enzyme to be identical, or at least certainly very nearly allied to, **trypsin**, since, like the latter, it furnishes **tyrosin**. J. Hjort (I.) succeeded in detecting the presence of similar enzymes, capable of digesting fibrin, in various higher fungi; they afterwards completely degrade the peptone formed, from the above substance, along with leucin and tyrosin. Egg albumen is also attacked at the ordinary temperature. Zopf (X.) has published an observation on fungi capable of dissolving horny matter (keratin).

Diastatic enzymes, *i.e.*, such as are capable of converting starch into sugar, are also very often (§ 117) found in *Eumycetes*. Many species of the latter are specially productive in this respect, and are employed on this account in the fermentation industries; this is particularly the case with the species of *Mucor* that will be described in § 240, and the Koji fungus (*Aspergillus oryzæ*) dealt with in the last section but one. At present we shall not treat of the technico-mycological side of this faculty, but merely make a remark of a general physiological character, namely, with regard to the dependence of the formation of this enzyme on the external conditions. In this connection W. Pfeffer (V.) and

Jul. Katz (I.) examined *Penicillium glaucum*, *Aspergillus niger*, and *Bacillus megatherium*, and found that these two *Eumycetes* produce diastase, even in the absence of starch, provided no adverse influence comes into play. Such an influence has been traced to the presence of various sugars, in the case of *Penicillium glaucum*, the formation of diastase at medium temperatures ceasing when the (otherwise identical) nutrient media received an addition of either 1.5 per cent. of saccharose, 2 per cent. of grape sugar, or 10 per cent. of galactose. The secretion was also retarded by 5 per cent. of maltose. Enriching the nutrient properties of the medium is succeeded by an increase in the limit of the foregoing additions. In the case of *Aspergillus niger*, an addition of even 30 per cent. of saccharose merely restricts the formation of diastase (at 31.5° C.) without suppressing it altogether. The amount of diastase secreted per unit of time by this fungus was found to be greater (other conditions being equal) in the case of cultures in which provision had been made for the immediate separation of the resulting diastase by additions of tannin.

Enzymes (lipases—Hanriot (I.)—or steapsines—W. Biedermann (I.)) **capable of decomposing fats**, are found not only in the pancreas, blood, and other corporeal fluids of all animals hitherto examined, but also in the vegetable kingdom. Their occurrence in *Eumycetes* was first detected in the case of *Penicillium glaucum*, the discovery being made by E. Gérard (II.) and L. Camus (I.). They were then found by the last-named (II.) in *Aspergillus niger*, and by R. H. Biffen (I.) in an unidentified fungus infesting the cocoanut. J. Hanus and A. Stocky (I.) reported having observed the production of a similar enzyme by a number of mould fungi in the course of their investigations —referred to in a later section—on mouldiness in butter. It is not improbable that several varieties of lipases exist in the fungoid kingdom. The faculty of producing such an enzyme is of particular importance when the nutrient medium is rich in fat, *e.g.*, in the case of fungoid parasites infesting the fatty matter of insects. This faculty may be regarded as non-essential to such fungi as make their habitat on oil-cake (§ 235) and consume the fat therein, since—as has been shown, particularly by W. Sigmund (I.)—the oil seeds frequently contain lipase, and it is very probable that some portion of this is left behind in the oil-cake. It is, however, doubtful whether this residue has not already lost its power, and is consequently incapable of being utilised by the infesting organisms, the lipases being very sensitive to injurious influences (acids, salts, &c.). For this reason, it may be remarked in passing, all attempts at their isolation in a pure state have failed. The capacity of producing fat-decomposing enzymes is certainly possessed by the fungi which, within a few months, reduce the fat content in the mud of settling tanks in waste-water purifying plant, from over 20 per cent. to almost nil. Sundry highly instructive experiments on this point have been conducted by J. Bechold (I.).

With regard to the various enzymes of yeast in particular, full details will be given in a later section.

SECTION XI.

FERMENTATION BY ZYGOMYCETES.

CHAPTER XLIII.

MORPHOLOGY AND SYSTEMATOLOGY OF THE MUCORS.

§ 234.—Subdivision of the Order of Zygomycetes.

The Order of *Zygomycetes* (§ 221) is characterised by the capacity of forming **zygospores**. According as these resting cells are able to envelop themselves in a more or less completely developed capsule, or remain naked, the corresponding **Zygomycetes** are divided into two sub-groups. The higher of these, viz., the one forming the spore capsules, has received the name "**carposporangial**," from Brefeld, who united the whole of the members into a family, a few particulars of which will be given in § 237. Antithetical to these are the remaining, or "**exosporangial**," *Zygomycetes*, as they were termed by Brefeld. It is with this (larger) sub-group, the sporogenic organs of which remain naked, that we have first to deal.

The occurrence of several **methods of fructification** in one and the same species of fungus has already been mentioned in § 223, and is specially prevalent among the *Zygomycetes*. Apart from the characteristic zygospores, they are able to ensure the reproduction of the individual in other ways, special aptitude in this direction being exhibited by the genus *Choanephora*, which, in addition to zygospores and gemmæ, also puts forth conidia and endospores. In all other *Zygomycetes* only one or other of the two last-named methods of fructification is exhibited by any one individual. Sporangial fructification is characteristic of the *Mucor* family, a number of examples of which will be more closely considered later on.

The other *Zygomycetes*, on the contrary, exhibit conidial fructification, and are divided into three families, which do not come directly within the scope of the present work, and will therefore be dismissed with a few brief explanatory remarks. The family of the **Entomophthoreæ** forms a connecting-link between the orders of *Zygomycetes* and *Oomycetes*. The species of this family are almost exclusively parasitic on living organisms (insects, fungi, ferns), and one of them will, from its effects at any rate, be, superficially, known to the reader, namely, *Empusa muscæ*, the cause of a disease attacking the house-fly in late summer and autumn. From this cause numbers of these insects are found to adhere, straddle-legged, to walls and windows, and become surrounded by a distinct white aureole, consisting of unicellular conidia dispersed by the conidiophores protruding from the body of the insect. These conidia germinate into a structure similar to a budding mycelium (§ 219); and it is owing to this faculty that the wraith of the house-fly has for so long made its appearance in the controversy on the origin of the *Saccharomycetes* (§ 344).

The second of the three families of conidiophorous *Zygomycetes*, namely, the

Piptocephalideæ, on the other hand, lacks the faculty of producing budding cells (like the third family), and is distinguishable by the feature that the zygospore apparatus consists of five cells instead of three, as also by the fact that the conidia are multicellular. The members of this family are parasitic on various species of *Mucor*, to the hyphæ of which they attach themselves by means of special organs (haustoria), and then penetrate the interior for the purpose of abstracting nourishment. This behaviour they exhibit in common with the species of the third family of conidiophorous *Zygomycetes*, namely, the **Chætocladiaceæ**, whose conidia are unicellular and may be regarded as sporangia, whilst the contents are united to form a single endospore, instead of being divided into several. By this feature they reveal the connection, as regards progress of development, between conidial and sporangial fructification.

We can now collect the above particulars into the form of a

Schematic Subdivision of the Order of Zygomycetes.

- **Zygomycetes:** *Phycomycetes* capable of producing zygospores
  - (*a*) Naked zygospores
    - (α) Exhibiting sporangial fructification, but lacking conidial fructification . . . *Mucoraceæ.*
    - (β) Exhibiting conidial fructification, but lacking sporangial fructification . . . *Entomophthoreæ.* / *Piptocephalideæ.* / *Chætocladiaceæ.*
    - (γ) Exhibiting both sporangial and conidial fructification . . . . *Choanephoreæ.*
  - (*b*) Zygospores enclosed in a capsule . . . . *Carposporangial Zygomycetes.*

Of these six families, only the first and last will be dealt with in the following paragraphs, chiefly from the standpoint of Physiology and the technology of fermentation, leaving out of consideration their morphology and development except in so far as mention of these is absolutely essential to our purpose. Readers desirous of obtaining fuller information on the two latter points are referred to Alfred Fischer's (III.) monograph on the *Phycomycetes* and *Zygomycetes*.

§ 235.—Subdivision of the Mucor Family.

From the explanations in the preceding paragraphs, the *Mucoraceæ* may be defined as *Zygomycetes* which exhibit sporangial, but not conidial, fructification, and produce naked zygospores. This family may be subdivided into three sub-families, of which, however, only one falls within the scope of the present work, whilst the others will merely be referred to in order to facilitate comprehension of the connection existing between them.

The sub-family of **Thamnidieæ** is characterised by the possession of two kinds of sporangia; on the one hand, a large, normal, polysporous sporangium, on the crown of the sporangiophore (terminal sporangium); and, on the other hand, certain far smaller sporangia, which are situated lower down and put forth by whorled lateral branches of the sporangiophore. This second kind of sporangia are destitute of columella, contain only a pair of endospores—sometimes only a single one—and are known as sporangioles. The most closely investigated member of this sub-family, *Thamnidium elegans*, is shown in Fig. 111. Starting from observations conducted by Brefeld (IX.), the dependence of fructification, in this species, on the external conditions (chemical composition and concentration of the nutrient substratum; temperature) was examined by J. Bachmann (I.). This worker demonstrated that, by controlling these conditions, it is possible to compel the fungus to produce either terminal sporangia or sporangioles exclusively, or both together, or again to remain barren of fruit.

The second sub-family of the *Mucoraceæ*, namely, the **Piloboleæ**, is distin-

guished by the feature that the ripe sporangium—owing to its peculiar structure—is released, and even forcibly expelled from, the organ on which it has been developed. Of the species belonging to this group, mention may be made of *Pilobolus crystallinus*, which is often found on horse-droppings.

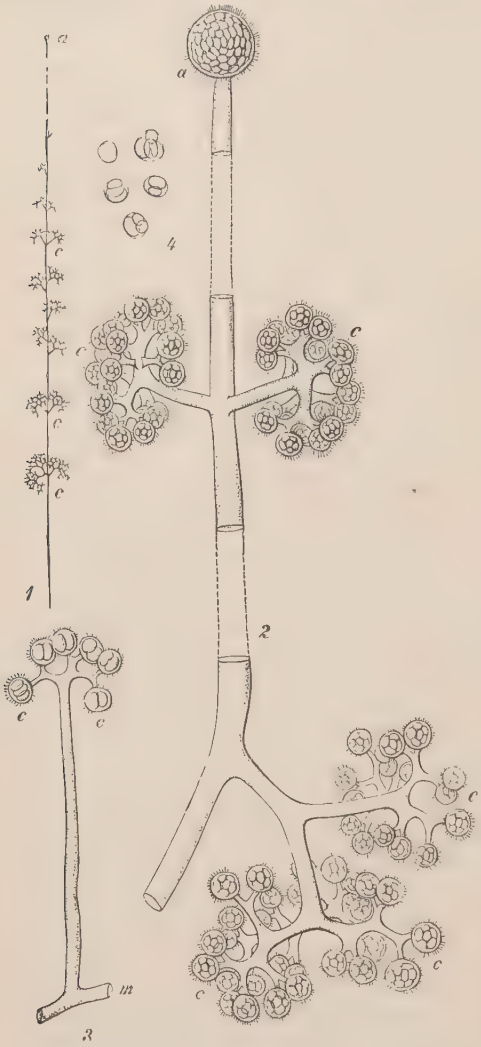


FIG. 111.—Thamnidium elegans, Link.

1. Sporangiophore, slightly (6) magnified;
2. Three pieces of same, more highly (120) magnified; *a*, terminal sporangium; *c*, sporangioles.
3. Stunted sporangiophore, exhibiting only sporangioles. Magn. 200.
4. Sporangioles that have separated from the sporangiophore. Magn. 200. (*After Brefeld.*)

Finally, the **Mucoreæ** constitute the third sub-family. They are distinguishable from the first by producing only a single kind of sporangia, which, unlike those of the second sub-family, do not separate from the sporangiophore before discharging their contents, but remain attached thereto after bursting. The discharge is effected in consequence of either the liquefaction or brittleness of the membrane of the ripe sporangium.

One of the genera in this third family, viz., the genus *Sporodinia*, is characterised by the forked branchings of its sporangiophores and the suspensores of the zygospores. This form is represented in Fig. 107. On the other hand, in all the remaining *Mucoreæ*—which have been arranged into five genera by A. FISCHER (III.)—these organs are either not branched at all or at least not forked.

The genus *Phycomyces* is characterised by the possession of spinous prolongations on the suspensores and sporangiophores, which latter are unbranched, olive-green in colour, and possess metallic lustre.

One species of this genus, *Phycomyces nitens*, is plentifully met with in empty oil-casks, on oil-cakes, in concentrated fodder works, and similar places, and puts forth stiff, upright sporangiophores 7 to 30 cm. long and 50 to 150 μ in diameter. These become crowned with an initially orange, but finally black, globular sporangium, 0.25 to 1.0 mm. across, exhibiting a cylindrical columella and filled with ellipsoidal, yellow-brown, thick-walled endospores, 16 to 30 μ long and 8 to 15 μ broad. It has not yet been ascertained whether this species feeds on the fats present in the aforesaid medium; in fact, the whole question of the decom-

position of fats by mould fungi (§ 233), on which sundry observations have been communicated by R. H. Schmidt (I.) and by Ritthausen and Baumann (II.), is a matter still requiring closer attention.

The genus *Mucor* is distinguished by the absence of spiny branches on the suspensores, by the silky gloss of the sporangiophores, and by the liquefaction of the ripe sporangium membrane. The contents of the ripe sporangium are only partly consumed in building up the spores, the remainder serving as a matrix wherein the individual ripe spores are embedded and separated from each other. Now this matrix is capable of distension, and holds the crowd of spores together, even in microscopical preparations; whereas on the other hand the sporangial membrane, being liquefiable, is either quite invisible in such (aqueous) preparations, or at most is only seen as a residual trace at the point of attachment to the sporangiophore. An attempt is made to portray this in Fig. 112. The outside of the sporangial membrane is found to be more or less closely set with crystals of calcium oxalate. A few of the species composing this genus are pathogenic, and therefore interesting to the pathologist, since they are able to set up mycosis (in this case *Mucor-mycosis*) or fungification of the body they have infested, or into which they have been artificially inoculated. Of these pathogenic species of Mucor—some of which have not yet been properly examined and classified in a botanical sense—mention may be made of the following: *Mucor pusillus*, discovered by Lindt; *Mucor corymbifer*, examined by Lichtheim, and which is probably identical with Lindt's *Mucor racemosus*, subsequently also named *Rhizopus ramosus*; finally Siebenmann's *Mucor septatus*, which is perhaps the same as *Mucor racemosus*. These pathogenic species have all been tried on warm-blooded animals, and therefore thrive at incubation temperature.

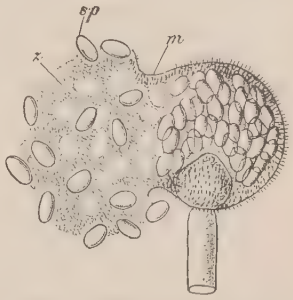


Fig. 112.—Mucor mucilagineus, Brefeld.

Newly burst sporangium. *m* is the membrane, *z* the matrix, *sp* the spores. Several of the latter have been squeezed out of the sporangium. Magn. 300. (*After Brefeld.*)

§ 236.—The Genus Mucor

was established by Micheli as far back as 1729. During the succeeding 140 years it received attention at the hands of a large number of workers, and a considerable number of species have been described—for the most part imperfectly. The species of this genus may be divided into three groups. One of them comprises all the species with **unbranched sporangiophores**, the chief representative being *Mucor mucedo*, with which is associated *M. mucilagineus*, &c. The species of the second group may exhibit **clustered branchings** of the sporangiophores; they include *Mucor racemosus*, *M. erectus*, *M. tenuis*, *M. fragilis*, *M. corymbifer*, and *M. pusillus*. Finally, the characteristic feature of the third group is a more or less decided **sympodial branching** of the sporangiophore. This group comprises *Mucor spinosus*, *M. circinelloides*, *M. alternans*, as well as the *M. Rouxii*, *M. javanicus*, &c., described in § 240. The former will now be described from a morphological standpoint, their physiological characteristics being deferred for consideration until § 239.

Mucor mucedo is the oldest known species, and was first fully described by O. Brefeld (I.) in 1872. The development of its mycelium has already been diagrammatically illustrated on p. 2. From the mycelium itself arise stiff sporangiophores, 30 to 40 μ thick and (according to the conditions of growth) 2 to 15 cm. in height. The apex of each supports a single globular sporangium,

which is closely covered with fine needles of calcium oxalate, and usually measures 100 to 200 μ in diameter, though, as shown in Fig. 113, it may be much smaller under unfavourable conditions. The spores, which are in the shape of an elongated ellipsoid, and about twice as long as they are broad, may differ in size in one and the same sporangium, the usual measurements, however, being 6 to 12 μ and 3 to 6 μ respectively. The cell contents are faint yellow in colour, the membrane colourless and smooth. The zygospores, the gradual development of which is represented in Fig 101, germinate by putting forth direct an unbranched sporangiophore with an attached sporangium. This species does not produce gemmæ.

A sporangium of *Mucor mucilagineus*—which was first mentioned by Brefeld (IV.)—is shown in Fig. 112, which also reveals the considerable dimensions of the spores. These are oval, 30 to 33 μ long and 15 μ broad. It has an affinity for horse-droppings, and is revealed, in cultures on this medium, by the surface of the sporangiophore being thickly covered with fine drops, as though bedewed.

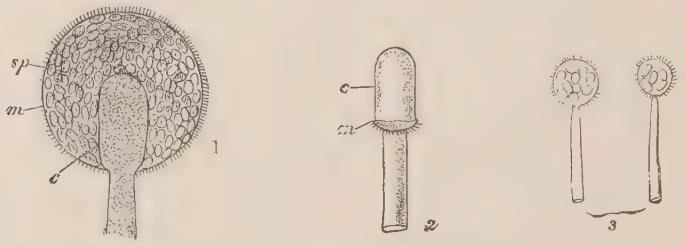


Fig. 113.—Mucor mucedo, Brefeld.

1. Sporangium of normal dimensions. *m*, the membrane; *sp*, the spores; *c*, the columella. Magn. 225.
2. Residue of an emptied sporangium. Only a fragment (*m*) of the membrane is left on its point of attachment to the sporangiophore; *c* is the columella. Magn. 180.
3. Stunted dwarf sporangia, with only a few spores and devoid of columella. Taken from an individual infested with a parasitic fungus (Piptocephalis). Magn. 300. (*After Brefeld.*)

Mucor racemosus Fresenius exhibits not merely the clustered branching of the sporangiophore, shown in Fig. 114, and a somewhat different development of the columella, but also the formation of chlamydospores already mentioned in § 223. These are not peculiar to this species, but are found on several allied kinds, though in others they are entirely lacking. Consequently, Brefeld (VIII.), to whom we are indebted for thoroughgoing researches on this point, united the former into a new genus, *Chlamydomucor;* and on this account *Mucor racemosus* also appears in the literature, under the synonymous designation *Chlamydomucor racemosus*. It produces sporangiophores, which, according to the environment, measure 5 to 40 mm. in height and 8 to 20 μ thick. Each of the several branches is crowned with a sporangium, 20 to 70 μ in diameter. The spores are globular, or of short ellipsoid form, and measure 6 to 10 μ in length by 5 to 8 μ in breadth. Fuller particulars respecting allied species, many of which are probably nothing but sports, will be given in §§ 240 and 241.

Mucor erectus Bainier has already been mentioned (§ 221) as an instance of a fungus forming azygospores in addition to zygospores. As there shown in two figures, sporogenation proceeds in this species in a manner similar to the typical formation of zygospores, in so far as two opposite gametes are produced.

This procedure is more decidedly evident in the case of *Mucor tenuis Bainier*, there being no approximation of the pairs of sporangiophores. The azygospores are formed, singly and independently, on the apices of branches of an erect sporangiophore, arising from the nutrient substratum (or the mycelium), and

which therefore assumes a clustered appearance. It is represented in Fig. 115. On the other hand, normal zygospores have not hitherto been discovered on this fungus.

Mucor fragilis, Bainer, resembles the foregoing species as regards the structure of the sporangium; except that the latter is smaller. The same applies also to the spores, which are oval in shape, about 4 μ in length, and 2 μ in breadth.

Mucor spinosus, van Tieghem, owes its specific name to the spiny projections, exhibited, to the number of about a dozen, on the crown of the cylindrical or pear-shaped columella. In preparations submerged in water, the membrane quickly dissolves and vanishes from sight, leaving a picture recalling that of the head of a conidiophore of any *Aspergillus*. On this account the fungus was at one time called *Mucor aspergilloides*, though the name has now gone out of use.

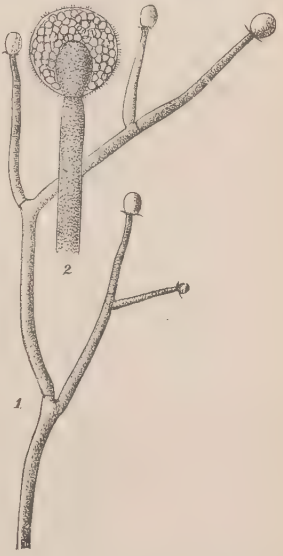


FIG. 114.—Chlamydomucor racemosus, Brefeld.

1. Branched sporangiophore. Magn. 80.
2. Optical section of sporangium, more highly magnified (300 times). (*After Brefeld.*)

Mucor circinelloides, van Tieghem, and *Mucor alternans*, van Tieghem, are very similiar in a morphological sense, particulars on which point will be found detailed by ALF. FISCHER (III.). The former of the two has been drawn by GAYON (VI.), and the other by GAYON and DUBOURG (III.). Morphologically, both species form connecting-links between the allied genera *Mucor* and *Circinella*, which latter differs chiefly from the former by the drooping manner in which the sporangia overhang and by the resistance of their membrane.

Mucor piriformis, which was first discovered (on rotten apples) and described by ALFRED FISCHER (III.), was so called on account of the pear-like shape of the columella, the broader (about 140 to 280 μ) upper end of which extends a distance of about 200 to 300 μ within the sporangium. When light is admitted to cultures of this fungus, grown in a suitable nutrient solution, it elaborates comparatively large quantities of citric acid from sugars, as was pointed out by C. WEHMER (X.). In fact a patent has been taken out in Germany (No. 91,891) for a process of manufacturing citric acid by this means. The subject will be mentioned again in a later chapter.

§ 237.—Rhizopeæ.

The higher, or **carposporangial, group** of the *Zygomycetes* (§ 234) can be divided into two families: **Rhizopeæ** and **Mortierellæ**. The former of these exhibits in a lower degree a peculiarity more highly developed in the second family, namely, the formation of an integument which surrounds the sporogenic organ and therefore raises it to the dignity of a true spore fruit. This second family does not come within our purview, and all that need be said about it is that all its species, especially *Mortierella Rostafinskii*—which occurs on horse-dung and was discovered and closely examined by BREFELD (IV. and IX.)—exhibit this method of fructification. From the earliest period of their development the zygospores are surrounded by a progressively increasing network of hyphæ, which spring from the adjacent mycelium, branch extensively, and finally weave themselves into a felted capsule surrounding the zygospore on all sides.

A less extensive development is exhibited by the integument, which endeavours to envelop the sporangium ; it does not increase to more than a dense cluster of hyphæ, which surround the lower end of the sporangiophore, without, however, being able to reach the sporangium itself. A view of this is given in Fig 116.

In the family *Rhizopeæ*, however, which comprises the two genera *Rhizopus* and *Absidia*, this integument of the zygospores is either absent (*Rhizopus*) or

Fig. 115.—Mucor tenuis, Bainier.

Azygospores in various stages of development. (*After Bainier.*)

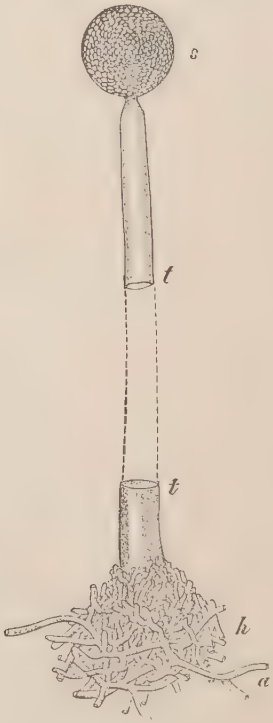


Fig. 116.—Mortierella Rostafinskii, Brefeld.

The lower end of the sporangiophore (*t*) is enveloped by a plexus of hyphæ (*h*), which have proceeded from the stolon (*a*). Magn. 100. (*After Brefeld.*)

only slightly developed (*Absidia*), being arrested at the stage of spiny processes protruding from the suspensores. The special position of this family, outside the exosporangial *Zygomycetes* (§ 234), forming as it does the connecting-link between them and the *Mortierella*, cannot therefore be founded on this indefinite characteristic of integumentation, but rather depends on the property it shares with the family just mentioned, namely, the development of stolons and the resulting peculiar conformation of the thallus. Whereas in the *Mucoreæ*, which closely resemble the *Rhizopeæ* in many other respects, the sporangiophores spring from parts of the mycelium that are not in any way specially distinguishable from the rest, there is developed between these two organs, in the *Mortierella* and *Rhizopeæ*, an intermediate organ which determines the location of the sporangiophore. This is effected in the following manner : a hypha, which, from its peculiarities, is called a **stolon**, begins to project outward from the mycelium growing in and upon the nutrient substratum. Describing a considerable curve,

this stolon endeavours to make its way to a substratum, which may either be a distant portion of the nutrient medium, or the walls of the vessel in which the latter is contained. The peculiar nutational movement described by the apex of the growing stolon was more closely examined by J. Wortmann (XIV.) in the case of *Rhizopus nigricans*. On coming into contact with the desired substratum, the stolon first endeavours to attach itself thereto by putting forth a system of short hyphæ, which fit close against the substratum, and on account of their branched and root-like form are termed **rhizoids**. In their entirety they

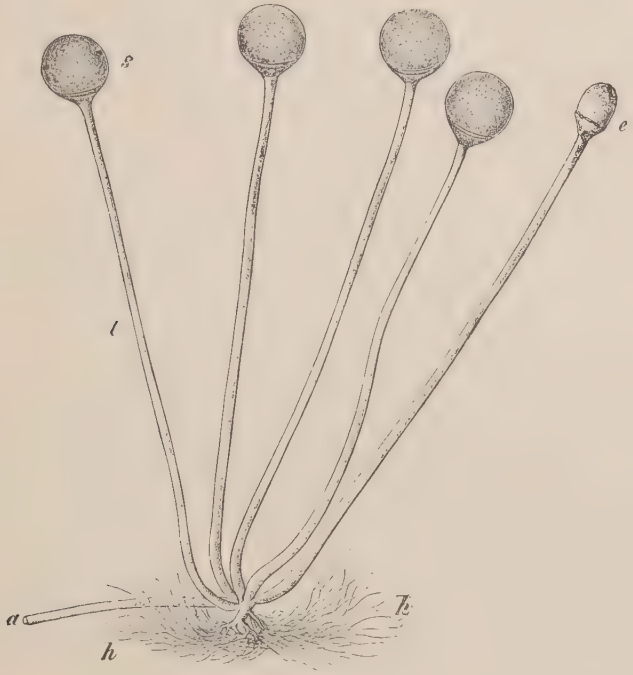


Fig. 117.—Rhizopus nigricans, Ehrenberg.

(*a*) is the extremity of a stolon, which has developed into the appressorium (*h*). This latter is the starting-point of the sporangiophores (*t*), four of which are shown with the sporangia (*s*) unbroken, whilst the columella (*e*) is all that remains of the fifth. Magn. 30. (*After Brefeld.*)

constitute an adhesive organ, called an **appressorium**, which is copiously supplied by the stolon with nutrient materials from the distant mycelium, and in turn puts forth stolons of the second order. These now extend to farther distances and produce appressoria of the second order, which form the starting-point for stolons of the third order, and so on; and in this manner the vicinity of the nutrient substratum is overrun with a fine network extending over a considerable area. It is from this network, and not from the mycelium itself, that the sporangiophores proceed, springing from well-defined centres. Thus, in the case of the genus *Rhizopus*, the sporangiophores take their rise exclusively from the appressoria, from each of which spring usually three to five, more rarely up to as many as ten. In the genus *Absidia*, on the other hand, the sporangiophores spring from the crown of the arch formed by the curved stolon.

The development of appressoria is a consequence of the mechanical attraction exerted on the tip of the stolon by the objects with which it comes in contact.

If means be taken to prevent the occurrence of such contact, for example, by compelling the stolon to grow vertically downwards, the stolon itself will develop into a sporangiophore direct. Instead of forming an appressorium, the apex—as J. WORTMANN (XIV.) has shown in the case of *Rhizopus nigricans*—develops into a normal sporangium. Another instance, in the fungoid kingdom, of the effect of such contact attraction—which is also frequently observed in the higher plants, especially in the curvature of tendrils—is met with in *Phycomyces nitens*, and was first noticed by ERRERA (VIII.), but afterwards more fully investigated by WORTMANN (XIII.). The sporangiophores of this *Mucorea*, when gently touched at the side, curve in such a manner that the place touched assumes a concave form. This phenomenon is termed **haptotropism**. We are indebted to A. DE BARY (II.) for the earliest observations on the relation between contact attraction and the formation of appressoria in fungi. The matter afterwards engaged the attention of M. BÜSGEN (II.)—in the case of *Botrytis cinerea*—and others.

Rhizopus nigricans is the best and longest known member of this family. In 1818 it was described by EHRENBERG under the name *Mucor stolonifer*, which is still used by several workers; and the same authority afterwards conferred on it the new appellation. This saprophyte readily and almost invariably infests vegetable substrata—*e.g.*, fruit—when the latter have been exposed to the air for some time in a damp condition. The stolons often attain a length of 3 cm.; the sporangiophores may grow to a height of 4 mm. (Fig. 117). The sporangium is pure white at first, afterwards yellow, and finally almost black. When ripe, the membrane liquefies on coming in contact with water, and therefore ceases to be visible in ordinary microscopical preparations. In such case beginners generally mistake the large columella for the wall of the sporangium. The spores are of varied form, mostly oval, and their longitudinal measurement is 6 to 17 μ.

The name *Rhizopus oryzæ* has been given by WENT and PRINSEN GEERLIGS (I.) to a fungus discovered by them in Ragi (§ 241), the sporangia and spore of which organism are considerably smaller than those of *R. nigricans*. Nevertheless, fluctuations in size are met with in both cases, according to the conditions of nutrition and development. This species was examined by C. WEHMER (XIV.), who failed, however, to find any fundamental difference between the same and *R. nigricans*; and it is therefore probably nothing more than a variety of this last fungus.

CHAPTER XLIV.

FERMENTATION BY MUCORS.

§ 238.—Intramolecular Respiration and Alcoholic Fermentation.

The exhalation of carbon dioxide, to which the name **respiration** has been given, may occur in both animal and vegetable cells, under two widely different sets of conditions: either in the presence and unrestricted access of oxygen, or in the entire absence of this gas. In the former case the process is termed oxygen respiration; but only a few remarks can here be made in connection therewith, for the most part to supplement the thorough description given in Pfeffer's (III.) *Handbook of Vegetable Physiology*. The volumetric ratio between the absorbed oxygen and the evolved carbon dioxide, or, as it is termed, the **quotient of respiration** ($CO_2 : O_2$), was found by Saussure, as long ago as 1833, to vary with the nature and actual condition of the plant under examination, as well as with the conditions of nutrition. With regard to this latter factor, it was shown by Diakonow (II.), in the case of *Penicillium glaucum*, that—other conditions being equal—the quotient of respiration is equal to unity when the foodstuff is sugar, but 2.9 when tartaric acid forms the nutrient substance. Puriewitsch (III.) then demonstrated, in the case of *Aspergillus niger*, that an increase in the concentration of the nutrient solution causes the quotient to rise at first, but afterwards to recede. The influence of temperature on the quotient of respiration was investigated by C. Gerber (I.) in cultures of the last-named fungus grown in Raulin's nutrient solution, with an addition of tartaric (malic or citric) acid, either alone or in conjunction with saccharose, as the organic foodstuff. Mention has already been made in § 233 of the influence of the degree of illumination on the quotient of respiration, as well as on a few other points in connection therewith.

We shall have to deal in a more thoroughgoing manner with the second kind of respiration, to which the name of **intramolecular respiration** has been given in consequence of a proposal made by Pflüger in 1875. This phenomenon consists of the persistent exhalation of carbon dioxide by cells excluded from a supply of free oxygen. The sole materials for this respiration are the carbohydrates, and in fact, primarily and directly, only certain kinds of sugar, which are decomposed in such a manner as to furnish, in addition to carbon dioxide, a residual product poorer in oxygen. This product, however, is by no means a uniform entity, but consists of a mixture of several substances, alcohol being an invariable constituent. In higher plants the energy with which carbon dioxide is liberated during intramolecular respiration is not infrequently on a par with that exhibited in normal respiration, and indeed is in some cases even superior; though, as has been shown by Wilson (I.), it is always inferior thereto in the case of fungi. All the preliminary conditions necessary to the inception of such a process of decomposition are present in the case of sweet (germ-free) fruits that are kept, uninjured, for a long time out of contact with air; and, in fact, it was in connection with such fruits that the first observations on intramolecular respiration were made, early in the nineteenth century. At that time the phenomenon was known as the **autofermentation of fruit** (*see* § 20). The extensive literature of

this subject has been collected in a meritorious treatise by C. Gerber (I.). It is not within the province of the present work to go more closely into the botanico-physiological aspect of this phenomenon; and besides it will be found thoroughly reported in the above-mentioned *Handbook* of Pfeffer's, by all who are interested in the matter.

The first observation of intramolecular respiration in fungi was that of Bail (I.), who, in 1857, showed that the mycelium of certain (unspecified) *Mucors*, when kept submerged in a saccharine liquid and out of contact with air, assumes an unusual form (*see* § 219), and instead of consuming the sugar to carbon dioxide, as it does in presence of air, converts the same in such a manner as to produce alcohol. This new fact was seized upon by Pasteur (II.), who endeavoured to utilise it as a pillar of support for his novel theory of fermentation. This we have already referred to in § 16 and § 113, mentioning at the time that it is not universally applicable. In fact, it is only unconditionally true in the case of a single group of phenomena, namely, those produced by strictly anaerobic agents, the butyric-acid bacteria in particular. On the other hand, difficulties are encountered in the case of alcoholic fermentation, Nägeli (III.) having shown that this fermentation, when excited by yeasts, proceeds just as well, or even better, in presence of free oxygen as when that gas is excluded—a result entirely opposed to the theory in question. Nevertheless, the observations of Pasteur and his predecessors, concerning the production of alcohol by *Mucoreæ* under the specified conditions, remain unimpeached; only, this formation of alcohol must not be regarded as perfectly identical in nature with the alcoholic fermentation excited by yeasts. Mention will be made further on respecting the difference between the two processes.

As already hinted, the presence and accessibility of certain kinds of sugar must be regarded as an indispensable preliminary condition for the occurrence of intramolecular respiration; and this will be more fully elucidated in subsequent paragraphs. Even under otherwise equal conditions, differences exist among the various fungi in respect of the extent to which intramolecular respiration may develop, and of the energy with which the resulting liberation of carbon dioxide proceeds. In some species the phenomenon seems almost entirely absent, and in others, *e.g.*, *Rhizopus nigricans*, it is very feeble. According to Diakonow (I.), *Penicillium glaucum* and *Aspergillus niger* cease to give off carbon dioxide very soon after the exclusion of air has been effected, and they perish within an hour, even when grown in nutrient solutions containing sugar. In this case, as was shown by Pasteur (III.) and Brefeld (XIII.), merely extremely minute traces of alcohol are formed. According to a private communication from Prof. Hansen, Schiönning has failed to confirm the contrary report of Elfving (I.) to the effect that he found considerable amounts (up to 4.2 per cent. by vol.) of alcohol in cultures of the first-named fungus; consequently this report must undoubtedly be based on error. Apart from the true alcohol-yeasts, in the restricted sense of the term, the producers of the largest (percentage) quantities of alcohol are to be found among the *Mucoreæ*. The behaviour of these under the conditions inducing intramolecular respiration will be dealt with in the next paragraph.

We will now turn to the difference between intramolecular respiration and alcoholic fermentation, and ask ourselves whether this difference is one of principle or merely of degree. Those who incline to favour the latter hypothesis were able to advance many circumstances in its favour. The chief products (alcohol and carbon dioxide) and the subsidiary ones (glycerin and succinic acid) are the same in both decomposition processes. The objection raised by the opposite party, namely, that the ratio of transposition is different in intramolecular respiration, and that a smaller quantity of alcohol is formed per unit weight of sugar consumed, was disposed of by the discovery of Godlewski and

Polzeniusz (I.), in 1897, to the effect that peas stored out of contact with air produce 101 to 103 parts of alcohol to 100 parts of carbon dioxide exhaled, *i.e.*, nearly as much as the 104.5 per cent. that should result from alcoholic fermentation according to Gay-Lussac's equation. Nevertheless, so far as we are able to judge in the present state of our knowledge, there exist two fundamental differences between the processes in question. One of them has already been mentioned, namely, the inception of alcoholic fermentation by yeasts in presence of air; and the second will become apparent as the mechanics of the two phenomena are examined and compared. As observed in higher plants and *Mucoreæ*, intramolecular respiration is undoubtedly an intracellular process, a manifestation of vitality on the part of the cell as such, and therefore inseparable from the corpus of the cell. On the other hand, such a local limitation cannot be assumed in the case of alcoholic fermentation by yeast, since it has become known that this phenomenon is not due to the action of the cell *per se*, but to a contained enzyme that is able to exert its powers outside, and independent of, the cell in which it originated, and can incite the aforesaid decomposition of sugar when placed in a test-tube. Through the discovery of this new fact, the phenomenon of alcoholic fermentation by yeast has lost, at one blow, its character as a direct manifestation of vitality on the part of the yeast cell, and has been transferred to the category of enzymatic action. Consequently, those who defend the hypothesis that these two processes are identical in principle, and differ merely in degree, will have to seek for the concomitant action of a similar enzyme in intramolecular respiration as well. So long, however, as this has not been proved we must still uphold the assumption that, on the basis of facts —so far as these are known—a distinction must be drawn between the formation of alcohol by intramolecular respiration (proceeding entirely within the cells themselves) and the alcoholic fermentation excited by a yeast enzyme (which may also act outside the cells). Nevertheless, for the sake of simplicity, we may still continue to speak in the following paragraphs of alcoholic fermentation induced by *Mucoreæ*, provided the difference specified be borne constantly in mind.

§ 239.—Mucor Fermentations.

Bail, who discovered that alcohol is formed by intramolecular respiration in certain species of *Mucors*, did not further investigate the chemico-physiological aspect of the question. Furthermore, the researches of Pasteur (III.), the next labourer in this field, suffer from the defect that he did not work with pure cultures, without which here (as elsewhere) no reliable results can be obtained; and besides, these experiments were mainly undertaken for the sole purpose of supporting the new theory of fermentation. Hence Fitz (IX.) was really the first (in 1873) to examine the question from the chemical side, *Mucor racemosus* being the organism selected for the purpose. Then followed U. Gayon (V.), who in 1878 tested *Mucor spinosus* and *M. circinelloides* for the possession of the faculty in question. Undeniably pure cultures, however, were first employed by E. Ch. Hansen (VIII.), who in 1887 reported on his experiments with *Mucor mucedo*, *M. racemosus*, *M. erectus*, and *M. spinosus*. His discoveries were afterwards supplemented by other workers who will be mentioned later on.

The degree of **susceptibility towards alcohol** is an important factor in solving the question as to the suitability of these fungi for the purposes of the distiller, since it is this sensitivity which, other conditions being equal, determines the **attainable production of alcohol**. Another influential factor is the fermentative activity, *i.e.*, the duration of time in which a given quantity of alcohol is produced. A few reports on this point are collected below:

Mucor mucedo has produced :

According to	After	In	Alcohol
Pasteur (III.)	14 months	wort	1.8 % by weight
Brefeld (XV.)	—	—	2.6 % „
Fitz (XI.) at 30° C.	7 weeks	must	0.8 % „
Hansen (VIII.) at 23° C.	15 days	wort	0.5 % by volume
and at room temperature	6 months	wort	3.0 % „

Mucor racemosus has produced :

According to	After	In	At	Alcohol
Fitz (IX.)	"many months"	must	25–26° C.	3.5–4.0 % by weight
Brefeld (XIII.)	—	nutrient soln.	15° C.	4.5 % „
Brefeld (XIII.)	—	—	high temp.	up to 5.5 % „
Pasteur (III.)	2 years	wort	room temp.	3.3 % „
Pasteur (III.)	2½ years	—	—	3.4 % „
Fitz (XI.)	1½ months	must	25–30° C.	2.3–2.7 % „
Hansen (VIII.)	12 months	wort	room temp.	7.0 % by volume

According to U. Gayon (V.) the production of alcohol by *Mucor spinosus* ceases as soon as the amount formed attains 1.5 to 2.0 per cent.; but Hansen, on the other hand, succeeded in detecting 5.4 per cent. by volume in wort cultures at 22° C., after 6½ months. *Mucor circinelloides*, according to Gayon (VI.), produces 5.5 per cent. of alcohol. *Mucor erectus*, according to Hansen, when grown in wort cultures at room temperature, furnishes 8 per cent. by volume in 2½ months, but only 7 per cent. at 25° C. It therefore probably forms an exception to the rule applying to all the other *Mucoreæ* mentioned, namely, that they produce large quantities of alcohol at higher temperatures than at lower. Thus, according to Fitz (IX.), *Mucor racemosus* is very sluggish in this respect at temperatures below 15° C. The weakest of all seems to be *Rhizopus nigricans*, which, according to Brefeld (XV.), ceases its activity as soon as 1.5 per cent. of alcohol has been formed. None of these species of Mucor can be used for distillery purposes, since they are far surpassed in competition by a number of true yeasts, which are capable of producing an equal quantity of alcohol in a much shorter time, and also to carry on the fermentation much further. With respect to a few other species, proposed and employed for this purpose, further particulars will be found in §§ 240 and 242.

Carbon dioxide, being the most important, in quantity, of the other products of intramolecular respiration in *Mucoreæ*, will be dealt with next. The reliable quantitative determinations of the production of this gas are very few in number, and for these we are mainly indebted to Fitz (IX.) and Gayon (VI.). The former worker, as the mean of nine experiments with *Mucor racemosus*, determined the ratio (by weight) of alcohol to carbon dioxide as 100 : 123.1; and Gayon obtained similar values for *Mucor circinelloides*, whereas, according to the Gay-Lussac equation, the ratio should be 100 : 95.6. The formation of aldehyde

and succinic acid in this process was first observed in cultures of *Mucor racemosus* by Fitz, and in those of *M. circinelloides* by Gayon, the last-named estimating the amount of this acid as 2.07 grms., and the amount of the resulting glycerin as 0.92 grm., per 100 grms. of saccharose consumed. These experiments with *Mucor racemosus* were afterwards resumed, with cultures of reliable purity, by O. Emmerling (III.), who obtained the following results from a culture grown for three weeks in a nutrient solution of saccharose and mineral salts, at 25° C., air being excluded: alcohol, 1.46 per cent.; succinic acid, 0.02 per cent.; glycerin, 0.12 per cent. The mutual ratio between these three products is 100 : 1.4 : 8.3. The amount of succinic acid seems to differ with different species; at least Brefeld (XIII.) states, on the basis of his experiments with *Rhizopus nigricans*, *Mucor mucedo*, and *M. racemosus*, that cultures of the first of these organisms yield the relatively largest proportion of acid, and those of the third the smallest, though still more than is furnished by beer yeast under similar conditions. These results presumably relate solely to succinic acid; at least this is probably the case with *M. racemosus*, since, according to Ph. Biourge (I.), this organism does not produce any volatile acids. Products of the ester class have often been observed to result from the intramolecular respiration of *Mucoreæ; e.g.*, an odour resembling that of pears, in cultures of *M. racemosus* by Brefeld, and one recalling that of plums, in *Mucor circinelloides* by Gayon.

We will now inquire as to the **kinds of carbohydrates** which may be drawn upon by the *Mucoreæ* for the purpose of forming alcohol; and we will divide these into sugars on the one hand and starch and dextrin on the other, so as to be able to classify the species of this genus on the basis of their practical applicability. Starch and dextrin do not yield alcohol direct, but only after they have been converted into sugar; consequently they can only come into consideration in the case of such *Mucors* as are capable of producing saccharifying enzymes. These species will be fully described in the next chapter, and for the present we will deal solely with the formation of alcohol from sugars, and with the inversion of the latter. So far as is known, **saccharose** is split up by only two of the species hitherto mentioned. One of these—according to Fitz (IX. and XI.), Brefeld (XV.), and Hansen (VIII.)—is *Mucor racemosus;* the other was observed by the last-named worker, and is probably nothing more than a sport of the same organism. These two species produce an inverting enzyme, which faculty constitutes a sharply defined distinction between them and the *Mucor* and *Rhizopus* species hitherto known. Hansen's comparative experiments revealed the importance of this physiological characteristic as a means of differentiating the individual species; and its value for the purpose in view is so much the greater from the fact that its occurrence in these two species, and absence in all the rest, is quite independent of the environment; which is more than can be said of morphological characteristics. It is therefore advisable to point out at once that saccharose cannot be inverted or fermented by *Mucor mucedo*, *M. erectus*, *M. spinosus*, *M. alternans*, *M. circinelloides*, *M. Rouxii*, or *Rhizopus nigricans*, though they can draw upon it as a source of carbon. On the other hand, with the exception of *Rhizopus nigricans*, they can all attack **maltose** and convert it into alcohol. Whether the fermentation is preceded by a fission of this disaccharide into hexoses, as occurs in fermentation with true yeasts, has not yet been investigated. None of them is capable of converting **galactose** into alcohol direct, though they can do so after inversion, as shown by Fitz (XI.) in the case of *Mucor racemosus*. This latter is therefore equally incapable with the other six species of producing an enzyme that can hydrolyse milk sugar. According to Dubourg (I.), **trehalose** can be fermented to alcohol by *Mucor alternans*, though **raffinose** cannot. From **dextrose** and **lævulose** each of the above-mentioned *Mucoreæ* can produce alcohol by intramolecular

respiration; and the same naturally also applies to the invert sugar formed from saccharose, by chemical reagents or the preparatory activity of other organisms. Presuming the above-mentioned preliminary hydrolysis of maltose to be correct, the formation of alcohol by the intramolecular respiration of *Mucoreæ* will fall under the same law as has already been found to prevail, without exception, in the alcoholic fermentation excited by *Saccharomycetes* and other budding fungi, namely, that the hexoses alone are capable of furnishing alcohol direct.

Basing on the one hand on the impregnability of saccharose, and on the susceptibility to dissociation of the hexoses on the other, Gayon (IV.) has attempted to elaborate a process for extracting sugar from molasses. The chief feature of the process is the decomposition, by *Mucor circinelloides*, of the invert sugar, which, as is well known, prevents the crystallisation of the accompanying saccharose in molasses; the resulting alcohol is then distilled off, and the saccharose is finally separated from the residue by crystallisation.

In the manufacture of snuff, a certain part seems to be played by the intramolecular respiration of *Mucors*, especially in connection with the after-fermentation in the so-called "pigtails" to which reference has already been made in § 105. Here, as also in tobacco pressed into casks, Miciol (I.) very often found *Mucor mucedo*, in addition to the *Sporodinia grandis*, at that time known as *Mucor flavidus*. J. Behrens (III.) afterwards found a second species (*Mucor racemosus*) both in the pigtails and on tobacco leaves, both green and dry; and he also observed that this latter organism acts therein as a producer of alcohol, drawing upon the sugar contained in the "sauces" (§ 105). On the other hand, he rarely succeeded in finding Mucor species on tobaccos that were not treated with "sauce."

With regard to the part played by Mucors in turning bread mouldy and in the rotting of fruit, this and correlative questions will be dealt with in the final section.

O. Johan-Olsen (II.), in describing the preparation and composition of Gammelost (a Norwegian cheese), states that certain species of *Mucor*—*Mucor casei*, and *Chlamydomucor casei*, both new species—take part in the ripening of this product. The experiments of Weigmann (X.) confirmed that, owing to their strongly developed capacity for degrading casein, fungi of this kind are able to take part in the ripening of cheese, and also to affect the flavour of the article by forming pungent fission products.

CHAPTER XLV.

THE USE OF MUCOREÆ IN THE SPIRIT INDUSTRY.

§ 240.—Mucor Rouxii and other Species of Amylomycetes.

The faculty of producing **diastatic enzymes**, and thereby exerting a saccharifying action on both starch and dextrin, is possessed by a considerable number of the Mucors. For the earliest information on this point we are indebted to U. Gayon and E. Dubourg (II.), who observed this faculty in *Mucor circinelloides* in 1886, and also found that this organism was only capable of exerting the influence specified when it developed in aggregations of gemmæ, but not when in the form of its normal mycelium. Shortly afterwards they reported (III.) on further similar experiments with *Mucor alternans*, and demonstrated that, under equal conditions, this organism converts starch into maltose and not into a hexose. The faculty is lacking in other species, *e.g.*, *Mucor racemosus*, which, according to Fitz (IX.), also leaves inulin unacted upon. There are other more powerful species of Mucors, which, on account of their practical importance for the spirit industry, will now be fully described.

For the preparation of rice spirit there is produced in China, Cochin China, and the neighbouring countries, an article known as **Chinese Yeast**, and put on the market in the form of flat mealy balls, about the size of a half-crown. Its preparation, composition, and application were first described in 1892 by E. Calmette (I.), whose reports were extended and supplemented by C. Eijkman (II.) in 1894. According to these authorities this so-called yeast is prepared by mixing equal quantities of husked rice—steeped in cold water—or rice meal and water, and pulverised aromatic drugs. The number of the latter varies, in different recipes, up to 46, but always includes garlic and galanga root. The resulting stiff paste is divided into small cakes of the shape mentioned above. These are laid on mats, previously coated with a thin but close layer of moistened rice husks (paddy), or are covered over with a layer of rice straw, and are kept in the dark for two to three days, at an air temperature of about 30° C. The balls, which by this time smell like yeast and have become coated with a fine white velvety covering, are next dried, either by exposure to the sun, or (in rainy weather) by gentle fire heat, and are then packed into bags for sale to the distillers. In India and China these balls are employed in the following manner: about $1\frac{1}{2}$ parts by weight of the finely crushed or ground mass are sprinkled over 100 parts of husked rice, that has previously been softened by boiling in water and cooled down sufficiently by spreading it out on mats. The two are intimately mixed together, placed in earthenware pots holding about $4\frac{1}{2}$ gallons (20 litres), so as to half fill the same, and are left covered up. The starch gradually saccharifies, and the mass consequently becomes partly liquid. At the end of about three days the pots are filled up with river water, and the sweet liquid is left to undergo alcoholic fermentation, which sets in very soon. This stage lasts two days, and the mash is then distilled in leaden retorts heated by direct fire. Each 100 kilos. of rice furnish about 60 litres of spirits, containing about 36 per cent. of alcohol.

The **flora of Chinese yeast** consists of bacteria, which, however, are of no

moment; then yeast cells, which must be regarded as the exciting agents of the alcoholic fermentation; and certain Mucors, which effect the saccharification of the starch. Of the last-named organisms, which alone concern us here, Calmette isolated a species, which, in honour of his teacher and colleague, E. Roux, he named *Amylomyces Rouxii*. He did not, however, ascertain anything definite respecting the morphology of this fungus and its position in the botanical system; but it was afterwards recognised as a Mucor by Eijkman, and its name changed to *Mucor amylomyces Rouxii*. The first thoroughgoing investigations regarding the progress of development of this organism were published by C. Wehmer (XI.) in 1900, who at the same time proposed to shorten the name to *Mucor Rouxii*. According to this worker the sporangiophore—which is about 1 mm. long and 7 to 14 μ broad—is generally branched, and in many cases is decidedly sympodial

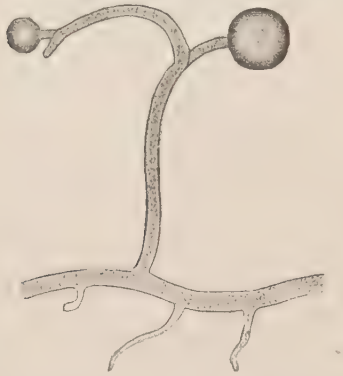


Fig. 118.—Mucor Rouxii.

Sympodial branched sporangiophore, from a 7-days old culture on sugar agar. Magn. about 160. (*After Wehmer.*)

Fig. 119.—Mucor Rouxii.

Sporangium almost entirely empty. Part of the wall still hanging in large shreds on the sporangiophore. In front of the globular columella are still lying three spores. From a culture on rice. Magn. about 230. (*After Wehmer.*)

(*see* Fig. 118). According to the conditions of nutrition the sporangia are either upright or declinate, globular, glass-clear and yellowish when ripe, and attain up to 50 μ in diameter. When they fall into decay a considerable remainder of the smooth, colourless membrane is left as a collar on the sporangiophore. The columella (Fig. 119) is globular, slightly flattened, smooth, colourless, and measures 23 to 32 μ by 20 to 28 μ. The spores are colourless, glabrous, and generally elongated (5 to 2.8 μ), rarely globular, and completely fill the sporangium. It is worthy of mention that the failure to form sporangia noticeable in many Phycomycetes is also observed here as a frequent occurrence, the sporangium, instead of ripening, either ceasing to develop more than half-way, or else germinating as a tube in place of forming spores. This phenomenon does not appear in cultures on rice, but most usually occurs in those on sugar agar, thus affording here a new and beautiful example of the influence of the conditions of nutrition on development. The mycelium produces an abundance of gemmæ, of different sizes and shapes, either globular (from 10 to 100 μ in diameter) or oval. They are colourless, or pale yellow to light brown, and have a very thick, smooth, colourless membrane. It is solely in the form of these resting cells that the fungus appears in Chinese yeast, to which, as Calmette has shown, it gains access from the rice husks and rice straw. This explains why, in many places, the manufacturers of Chinese yeast consider it indispensable to press a few moistened rice husks into each of the fresh, pasty yeast cakes. As soon as the gemmæ arrive in a suitable nutrient medium they put forth germi-

nating tubes and develop into mycelia. Up to the present no zygospores or yeast-like budding cells have been observed in this fungus. When grown on solid media (steamed rice in particular) the mycelium gradually assumes a highly characteristic orange colour due to golden yellow drops (oil ?) appearing in the cell, but only at room temperature, though not at 40° C. The optimum temperature for the development of the mycelium lies between 35° and 40° C. According to Calmette, a temperature of 75° C. kills the organism within half an hour, and 80° C. is fatal in fifteen minutes.

The same observer states that the diastatic enzyme produced by *Mucor Rouxii* exerts its most powerful effects at 35 to 38° C., and is destroyed on being warmed to 72° C. According to Boidin and Rolants (I.), the sugar thus formed from the starch is glucose. Like lævulose, saccharose, maltose, and lactose, this sugar serves as a source of carbon to the fungus, and is burnt off provided there is direct access of atmospheric oxygen to the mycelium. If, on the other hand, the latter is compelled to remain submerged in the nutrient substratum, the glucose is also converted into alcohol. A. Sitnikoff and W. Rommel (I.) state that the same also occurs in presence of *d*-mannose, fructose, galactose, trehalose, maltose, dextrin, and *α*-methyl glucoside, but not in presence of raffinose, lactose, saccharose, melibiose, xylose, arabinose, rhamnose, tagatose, *β*-methyl glucoside, inulin, &c. The inability of this fungus to produce invertin was recognised by J. Sanguineti (I.). This worker also performed a series of comparative experiments on the effective force and conditions of the diastatic enzymes produced by *Mucor Rouxii*, *M. alternans*, and *Aspergillus oryzæ*, and found that the last-named is weaker than the other two, so far as regards the saccharification of dextrin, the greatest power in this respect being exhibited by the second. The appearance of free acids in the cultures of *Mucor Rouxii* is also recorded by the same observer; Calmette supposes oxalic acid, Eijkman, on the contrary, lactic acid. The amount of acid may be so abundant as to kill the culture. The industrial utilisation of this fungus in Europe will be dealt with in the next paragraph but one. At present we will conclude with the remark that Boidin and Rolants' proposal to employ this fungus for converting into sugar, or alcohol, the dextrin left in the residues from distilleries operated on existing lines, would probably be too expensive outside the laboratory.

β-Amylomyces and *γ-Amylomyces* are the names of two species of *Mucor* which have recently found employment in the so-called **amylo process** (§ 242), instead of *Mucor Rouxii* (*α-Amylomyces*). Colette and Boidin found the *β* species on Japanese rice, the other (*γ-Amylomyces*) on Tonkin rice; and we are indebted to A. Sitnikoff and W. Rommel (I.) for fuller researches on their morphology and physiology. According to these workers, cultures of these two species on a nutrient solution containing glucose can be readily distinguished from those of *Mucor Rouxii*, by the circumstance that, whereas the latter develops as a weakly, barren mycelium almost entirely within the liquid, the *β* and *γ* species, on the other hand, also grow on the surface, forming well-developed aerial mycelia, which project above the level of the liquid a distance of as much as 3 cm., and bring forth sporangia. The latter often develop in a special manner. Instead of the tip of the sporangiophore gradually enlarging to a sporangium, the swelling here constitutes merely a preliminary stage. It does not itself develop into a sporangium, but puts forth one or more thread-like processes which may then become crowned with sporangia (one each). This is illustrated in Figs. 120 and 121. In both species the ripe sporangia are black, and of slightly flattened globular form, the transverse diameter varying between 45 and 95 *μ*, whilst the longitudinal diameter is about 15 *μ* smaller. The spores are oval or elliptical, brown in colour, and attain the following dimensions, in the dry state: *β* species, length about 9 *μ*, breadth about 5·7 *μ*;

γ species, 7 and 4 μ respectively. The formation of gemmæ on the mycelia is of very frequent occurrence, especially when air is excluded. The behaviour of these two fungi towards sugars was examined by the Lindner micro-fermentation method. The results showed that glucose, fructose, *d*-mannose, galactose,

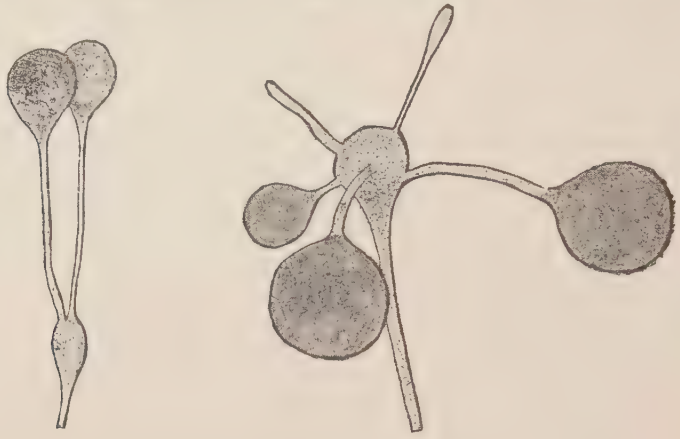


FIG. 120.—Sporangiation in β-Amylomyces.

The globular swelling at the apex of the hypha produces in the one case two branches, each carrying a sporangium, in the other case five branches, three of them exhibiting each a single sporangium in different stages of development. Magn. about 120. (*After Rommel's original drawing.*)

maltose, and dextrin are fermented by both these species, alcohol being formed; whilst, on the other hand, saccharose, melibiose, raffinose, and inulin are fermented by the β species solely, and trehalose by the γ species exclusively. The α- and the β-methyl glucoside remained unacted upon by either. Potato-starch paste, liquefied by an addition of nutrient salts, was saccharified more effectually

FIG. 121.—Sporangiation in γ-Amylomyces.

In the one example the knob-like swelling has put forth three branches, each with a sporangium; in the other, one of the branches is short and has no sporangium, the other two being long, declinate, and carrying sporangia. Magn. about 80. (*After Rommel's original drawing.*)

by each of these two species than by *Mucor Rouxii* in a comparative experiment. This activity has already been utilised in practice, and preference is given to the two new species in working the amylo process. In respect of energy in the production of alcohol they appear to be inferior to the other; but this does not lower their efficiency when used merely as saccharifying agents.

Mucor Cambodja, which was isolated from Chinese yeast and carefully examined by T. CURZASZCZ (I.), will produce 1.06 per cent. of alcohol in twenty

days in a nutrient solution containing 10 per cent. of dextrose. Sporangiation proceeds in a similar manner to the two last-named species of Amylomyces; but the organism differs from these by forming rhizoid processes, and therefore presumably belongs to the genus *Rhizopus*.

§ 241.—Ragi and Tapej.

The raw material employed in Java for the production of arrack consists of waste molasses from sugar-works, containing about 20 to 30 per cent. of glucose (or invert sugar) and 25 to 35 per cent. of saccharose. This molasses is reduced to about 15° Bé. (26.6° Ball.) by dilution with river water, and is then treated, in order to reliably accelerate alcoholic fermentation, with an auxiliary, which is comparable with the leaven (§ 148) used in European distilleries, and is known as **Tapej**. This is prepared from rice by the aid of a second auxiliary material, which the Malay natives of Java term **Ragi** or **Raggi**, and the Chinese settlers call **Peh-Khak**.

According to A. G. Vorderman (I.) this Ragi is prepared by crushing together pieces (rich in sugar) of the sugar-cane and the rootstock of galanga (*Alpinia galanga*), and making the product into a paste with rice meal. Went and Prinsen Geerligs (I.) state that garlic and other aromatic plants also form part of the mixture. The paste is divided into strips, which are dried in the sun, then broken up, mixed with water and lemon-juice, and left to stand for three days. The liquid portion is afterwards poured off, the coarser vegetable fragments are removed, and the pulpy residue is made up into flat, round cakes, about 4 cm. in diameter, which are left to dry and harden in the sun. Generally, however, they must first be laid in rice straw for a couple of days, or else a few fragments of fresh rice straw are kneaded in with the cakes. The dried Ragi cakes—which are sometimes called **Java yeast**—form an article of commerce in Java.

According to Eijkman (II.), Tapej, or, as it is also called, **Tsao**, is prepared by the aid of Ragi, by boiling husked Mochigome rice (*Oryza glutinosa*, known as "Ketan" in Java) in water till soft, spreading it out in a thin layer till cool, sprinkling it over with powdered Ragi (with which it is well mixed), and packing the whole into a cylindrical vessel (with a perforated bottom), which is then covered up with a mat and left alone for two days. At the end of this time the rice will be found changed into a semi-fluid, sour-sweet, coherent mass, which is now called Tapej. As already mentioned, it is used to excite fermentation in molasses. Its application will be again referred to in the next section.

The **flora** of Ragi, and of Tapej as well, comprises three groups of micro-organisms: first, bacteria, which need not be considered here; secondly, budding fungi, which will be dealt with in a later section; thirdly, fungi belonging to the family *Mucoraceæ*. The latter alone are concerned in the saccharification of the starch in Tapej, and with the liquefaction of the mass. Went and Geerligs (I.) discovered two forms of these in Ragi, and bestowed on them the names *Chlamydomucor oryzæ* and *Rhizopus oryzæ* (§ 237), but left open the question as to the probability of both forms belonging to one and the same species. One of them is in many respects similar to the *Amylomyces* (*Mucor*) *Rouxii* already described, and, like this latter, also has its habitat on rice straw. Nevertheless, certain differences have been observed between them, especially with regard to the absence of the faculty (possessed by the *Mucor* aforesaid) of producing alcohol from sugar when oxygen is excluded. In Ragi this species is found exclusively in the form of gemmæ, which are rich in glycogen, strongly refractive of light, and, according to C. Wehmer (IX.), retain their power of germination for more than a year and a half when in the dry state. In Tapej they then

germinate to a mycelium, which, however, rarely (and then only sparingly) succeeds in forming the (black) sporangia in that medium, though it will do so abundantly in pure cultures on boiled rice, &c. A certain interest attaches to the physiology of *Chlamydomucor oryzæ*. By the action of its diastatic enzyme it forms dextrose from starch. The amount of this sugar formed, however, depends on the kind of starch employed, the results obtained by Went and Prinsen Geerligs being: with Indian meal and potato flour, 8 per cent.; arrowroot, 16 per cent.; wheaten flour, 29 per cent.; ordinary rice, 44 per cent.; and Mochigome rice, 64 per cent. As is well known, the starch granules of this last-named seed consist mainly of amylodextrin, accompanied by a little erythrodextrin. It would therefore seem as though the fungus in question is only capable—preferably or perhaps exclusively—of saccharifying this carbohydrate, and not the granulose which forms the chief constituent of starch granules in the other kinds of meal specified. Hence this fungus might possibly be employed as an auxiliary in roughly estimating the percentage of granulose in starch. The yield of sugar obtainable by the action of this fungus on starch is somewhat low. Thus, for example, in an experiment recorded by Prinsen Geerligs (II.), 100 parts of amylodextrin furnished 110 parts of dextrose by saccharification under pressure with an acid, but only 68.5 of dextrose when saccharified by the fungus. The latter consumes a certain amount of the sugar as a source of carbon for its own needs; and it can also utilise alcohol for the same purpose. The nitrogen requisite for building up the cell it can take up from peptone, asparagin, and ammonium salts, but not from nitrates and nitrites. It is incapable of producing invertin.

A new species, allied to the foregoing, has been described, under the name *Mucor javanicus*, by C. Wehmer (XIII.). It occurs both in Ragi and Chinese yeast, mainly in the form of gemmæ, which retain their germinative power for at least five years in these media. Morphologically, they closely resemble *Mucor alternans* and *M. circinelloides*. The mycelial hyphæ measure 12 to 15 μ in thickness. With increasing age the presence of oil drops in the plasma imparts to the latter a decided coloration, which, however, is not so powerful as in the case of *Mucor Rouxii*. Similar fatty inclusions are also found in the gemmæ. These latter are put forth by the mycelium, both upon and in the nutrient substratum, and attain a diameter of 4 to 20 μ, being therefore far smaller than those of the last-named species. When the nutrient substratum is sufficiently consistent, there springs up from the mycelium a dense herbage of sporangiophores, which form sympodial branches, attain a height of 1 cm. and over, and develop a globular sporangium on each of their six or more branches. The dimensions of the sporangium increase with the height of the support and vary between the limits of 50 and 20 μ in diameter. The colour of the sporangium is yellowish grey to brownish; and the membrane is almost always smooth, as is also the globular (10 to 35 μ) columella. The spores are globular to elliptical, colourless, smooth and thin in the membrane, and measure 5 to 6 μ, or 5 to 7 μ by 4 to 5 μ. Up to the present, no zygospores have been observed. The optimum temperature of development lies between 35 and 40° C. When developed in saccharified malt extract or in a solution of dextrose and nutrient salts, this fungus produces an appreciable amount of alcohol; neither this matter, nor the capacity of the fungus for saccharifying starch, has, however, yet been thoroughly investigated.

Until further particulars are forthcoming, we must regard *Chlamydomucor oryzæ* as the principal agent in the diastatic process that goes on in the incipient Tapej. The gradually increasing quantity of sugar resulting from the activity of this fungus then furnishes the desired nutrient material for yeasts, which are either already present in the Ragi or have found their way—through the water or from the air—into the Tapej, where they reproduce freely and thus

impart to this material the property forming the object of its preparation: namely, to quickly excite fermentation in the dilute molasses into which it is introduced when ripe. Further particulars respecting the yeasts in Tapej and the application of this material will be given in a later section.

The natives of Java also prepare Tapej for another purpose. They express the sweet juice, allow it to thicken in the sun, and pack it in small twists made of Pisang leaves. Here it crystallises into crumbling masses, which are called Brem and furnish a favourite sweetmeat. The composition was ascertained to be in one case: dextrose, 69 per cent.; dextrin, 10.6 per cent.; ash, 1.2 per cent.; and water, 18.7 per cent. In addition, Tapej is also eaten alone by the natives.

§ 242.—The So-called Amylomyces Process.

The **Amylomyces process** (or **Amylo process** for short) is the name given to the process for the industrial utilisation of the diastatic activity of *Mucor Rouxii* and several allied fungi. A company, the "Société d'Amylo," was founded by A. Collette and A. Boidin (I.), who also, in 1897, took out in the name of this company a German patent for a "process for producing alcohol from starchy materials, by means of aseptic saccharification and fermentation with *Mucedineæ*, characterised by the feature that, in order to prevent the combustion of starch during aseptic saccharification by means of *Mucedineæ*, the sterilised raw material, treated with water, is subjected to the passage of a current of germ-free air, the material being meanwhile advantageously kept stirred by mechanical means; after which the air-supply is cut off and the material is pitched with yeast, which, by means of the consequent rapid liberation of carbon dioxide, prevents the combustion of the alcohol already formed by the *Mucedineæ*."

We are indebted to A. Fernbach (II.) for a lucid description of the practical performance of this process in the patentees' works, the maize distillery at Seclin near Lille. Of this description only the main points can be reproduced here. The maize, mixed with twice its own weight of water, is steamed for three hours, under a pressure of $3\frac{1}{2}$ to 4 atmospheres, in a Henze high-pressure steamer, and is then discharged into a preliminary mashing tun previously charged with 1 part, by weight, of green malt for every 100 parts of maize, and sufficient cold water to reduce the temperature of the whole to 70° C. Presumably the sole purpose of the added malt is to liquefy the swollen starch of the steamed maize, and thus render it more readily accessible to the saccharifying action of the fungus that is afterwards added. After an hour's stirring the mixture is transferred to an autoclave, where it is sterilised at 120° C., and whence it is forced, by pressure, into a cylindrical metallic fermenting tun, which is enclosed on all sides. In construction, this vessel resembles the apparatus used in breweries and distilleries for the production of pure-culture yeast, and therefore the intrusion of all extraneous germs is excluded with certainty. To sterilise the interior, a portion only of the sterilised wash is introduced at first and is treated with sulphuric acid, being afterwards boiled a short time by direct steam. By this means any germs that may be adhering to the upper parts of the inside are loosened by the condensed steam, and swilled down into the hot, acid mash, where they are quickly killed. The acid is said to be sufficiently neutralised by the lime present in the subsequently introduced remainder of the mash, which is delivered from the autoclave under pressure. The total capacity of the fermenting vessel, as used at Seclin, is about 23,800 galls. (1080 hectolitres), and the charge measures about 22,000 galls. (1000 hl.). At the present time this charge is prepared from about 18 tons (18,000 kilos.) of material, instead of 10 tons in the earlier days of the process. As soon as the fermenting vessel is charged,

the supply of steam is shut off, and sterilised air is then introduced, under pressure, through a special pipe, so as to keep the mash under pressure during the commencement of cooling, which soon begins. The cooling is effected by the aid of water, which is caused to trickle down the outside of the fermenting vessel from a perforated tube surrounding same. The escaping air (as also the carbon dioxide given off during fermentation) is removed through a pipe dipping into a water seal, to prevent direct connection with the atmosphere. By the end of five hours the temperature of the contents will have sunk to 38° C. They are then inoculated with a pure culture of the *Amylomyces*, rich in spores, contained in a 3-litre Pasteur flask charged with 100 c.c. of beer-wort and 100 c.c. of boiled rice; this leaven is introduced through a previously closed special pipe in the upper part of the fermenting vessel, which pipe is immediately recapped. The internal stirring apparatus, the shaft of which passes through a germ-tight stuffing-box, is next set in motion, and during the succeeding twenty-four hours a gentle current of air is passed through the mash, which at the same time is kept in motion to prevent the development of a mycelial herbage on the surface of the mash, since this would lead to a loss of material by brisk respiration.

In twenty-four hours after inoculation, the entire mash is permeated with the mycelium of the *Amylomyces*. The formation of sugar now proceeds with rapidity. Its fermentation, however, according to the patent in question, is not (or at most not to any considerable extent) effected by *Amylomycetes* capable of exercising this function, but rather by a suitable pure yeast, which at this stage is added to the mash cooled to 33° C., the amount taken being about 5 grams grown in about ½ a litre of nutrient solution in a Pasteur flask. The reproduction of this comparatively small sowing proceeds rapidly during the next twenty-four hours, thanks to the uninterrupted admission of air. The latter is then excluded, whereupon both species of fungi act conjointly during the three following days: the *Amylomyces* saccharifying the hitherto unconverted portions of starch and dextrin, whilst the fermentation of the sugar already present and that continually forming is chiefly effected by the yeast. The principal task assigned to the yeast by the patentees is not this fermentation, but merely the formation of carbon dioxide, thereby ensuring the presence of an oxygen-free atmosphere within the fermenting vessel, and thus precluding the consumption of the alcohol by the *Amylomyces*. Collette and Boidin (II.) afterwards, in 1898, took out an additional patent, in which it is explained that the yeast may be entirely dispensed with if the saccharified mash be freed from oxygen by stopping the supply of air immediately the iodine test reveals that the conversion of starch is completed; then passing carbon dioxide through the mash for an hour, and afterwards leaving the *Amylomyces* to proceed with its task alone. It is nevertheless a fact that yeast is still added in the Amylomyces process plant erected in Austria-Hungary since the date of this additional patent. An English patent for a mechanico-technological modification of the process was also taken out by Collette and Boidin (III.) in 1898.

An important simplification of this process is practised in the Anker Distillery at Antwerp, as reported by O. Saare (I.). In this case the sterilisation in the autoclave and the boiling with sulphuric acid are omitted. The mash, prepared from steamed maize with an addition of 2 per cent. of malt, is transferred direct to the fermenting vessel, where it is boiled for a short time by steam under ordinary pressure, then cooled and aerated, the inoculation with the *Amylomyces* culture being afterwards effected in the manner already recorded. When this has done its work and the mash no longer reacts with iodine, an addition of yeast is given, the kind used being the No. II. race of the Berlin Station (§ 245).

It is not the author's province now to pass judgment on the value of the Amylomyces process from a technological and economic standpoint, the more so because the reader interested in this matter will find more precise data in the review published by M. Delbrück (III.). The chief advantage of the Amylomyces process is the abolition of the expensive additions of malt requisite in the older method of saccharification, the amount formerly needed being up to 15 per cent. in the case of maize, and 2 to 3 per cent. in the case of potatoes. With regard to the yield furnished by the Amylomyces process, it is stated that in the Seclin works, 37.8 litres of absolute alcohol are obtained per 100 kilos. of maize containing 57.5 per cent. of starch, a yield corresponding to 66.2 litres per 100 kilos. of starch. Owing to the large proportion of mycelial hyphæ, the residue filters easily, a circumstance of considerable value in French distilleries, where the residues are not consumed on the premises for fodder, but are dried and pressed for further treatment.

Viewed from the mycological standpoint, the advantage, and at the same time a source of weakness, in this method lies in the inoculation with such a small amount of yeast, and therefore in the abolition of the preparation and employment of a yeast-mash (§ 148). In the present case the mash is deprived of the bactericidal lactic acid, and is therefore readily infested by injurious germs, should the latter be present at the start, or even find their way in as a result of carelessness in the act of inoculation, at a time when only a few individual organisms of the *Amylomyces* or yeast are present. On the other hand, it is claimed in favour of the raw spirit formed in this process that it is very low in fusel oils. Another question (of a purely economic character) is how far the extra output of alcohol is counterbalanced by the increased outlay in fuel for sterilising the mash, and by the interest on and depreciation of the necessary plant, which latter very soon wears out.

Finally, it should also be mentioned that, since 1898, the aforesaid patentees have replaced *Mucor* (*Amylomyces*) *Rouxii* by another species, namely, the so-called *β-Amylomyces*, or *Mucor β*, described in § 240. This organism is capable of dealing with more highly concentrated mashes than the other, and enables a charge of 25,000 kilos. of maize to be mashed to 1000 hl. (22,000 galls.) of goods. According to Boidin, a third species—namely, that already mentioned as *γ-Amylomyces*, or *Mucor γ*—is able to carry the fermentation 0.1 to 0.2 Balling further than is attainable by using the others aforesaid. Latterly, however, this fungus seems to have been abandoned again, since it was reported, at the commencement of 1900, that a Luxemburg grain distillery, working with the Amylomyces process, had superseded the foregoing species of *Mucor* by *Aspergillus oryzæ* (for description of which see a later section), and had by this means succeeded in producing 39 litres of alcohol per 100 kilos. of rye taken. It may also be mentioned that in this distillery the steaming, mashing, cooling, and fermentation were all carried out in one and the same vessel, specially constructed for the purpose. The aforesaid employment of this last-named fungus is also protected by a patent taken out by Collette and Boidin (IV.).

These same workers (V.) have also recommended the use of *Mucor Rouxii* for raising dough, especially in hot countries whither pressed yeast cannot be shipped. With this object they prepare the fungus in question according to a special process patented by themselves.

SECTION XII.

FORM, STRUCTURE, AND CHEMICAL COMPOSITION OF THE YEAST CELL.

CHAPTER XLVI.

MORPHOLOGY AND LIFE-HISTORY OF THE YEASTS.

§ 243.—Position of the Saccharomycetes in the Botanical System.

It was stated in § 220 that only a few classes of the sub-kingdom of Mycomycetes exhibit the faculty of producing endogenous spores, namely, the *Ascomycetes*. Consequently the latter may be defined as endosporogenic Mycomycetes. In the same place it was also mentioned that the organ wherein the endogenous spores are formed in this class is not called a sporangium but an **ascus**, and differs from the sporangium both as regards the greater fixity of shape and also the number, shape, and method of formation of the spores therein contained. These spores are therefore called **ascospores**.

The fundamental characteristic distinguishing the ascus from the sporangium resides in the behaviour of the bearer of the inherited properties of the organism, namely, the cell nucleus, during sporulation. The sporangium (p. 11) is polynuclear from the outset, whereas the ascus contains only a single nucleus at the commencement of sporulation. The nucleus—as will be more fully described in § 250—reproduces by subdivision into as many daughter-nuclei as there will be ascospores. In the sporangium, on the other hand, as many nuclei as there will subsequently be endospores are transferred from the aseptate mycelium to the sporangium before the separating membrane is formed.

It is only in the least highly developed *Ascomycetes* that the ascus springs direct from the mycelium; in all the rest it is developed in or upon a special organ called the **carpoascus**, and all the *Ascomycetes* fructifying in this manner are grouped into the sub-class of *Carpoasceæ*. Antithetical to this class are the lower *Ascomycetes*, the *Gymnoasceæ*, the asci of which are naked; and it is to this latter group that most of the technically important yeasts belong.

An examination of the development of the carpoasci of a number of species belonging to the first sub-class has revealed that here the asci are produced by a special organ, to which the name **ascogone** has been given. This is a unicellular or polycellular organ springing from the mycelium and putting forth the asci either direct or upon branched processes. At the same time it surrounds itself with an envelope of one or several layers, thus broadly completing the formation of the carpoascus. The shape of the envelope, and therefore of the entire carpoascus, forms the principal characteristic on which the sub-class of the *Carpoasceæ* are allotted their position in the botanical system. When the envelope does not enclose the ascogone on all sides, but merely serves as a support therefor (or for the asci) on the basal side, leaving the others free, then a disc-like or cup-shaped fruit is formed, which is called an **apothecium**. *Ascomycetes* producing

fruit of this kind are termed *Discomycetes*. If, on the other hand, the ascogones or asci remain surrounded on all sides by the envelope when ripe, then the fruit is termed a **perithecium**, *i.e.*, globular, oval, or in the form of a short bottle; and the *Ascomycetes* producing same are classed along with the order of *Pyrenomycetes*, or *nuclear fungi*, the second of the two orders into which the sub-class of *Carpoasceæ* has been divided.

If the perithecium is of a kind that exhibits no aperture at all during the whole period of its existence, so that the contained asci or ascospores cannot escape into the open air unless the perithecial membrane breaks up or is destroyed, then the perithecium is termed **cleistocarpous** or enclosed. All the *Pyrenomycetes* bearing fruit of this character are included in the sub-order of *Perisporiaceæ*. The common greenish blue bread-mould belongs hereto.

On the other hand, when the perithecium is of such a type that, in the ripening process, it develops an orifice through which the asci or ascospores are able to escape, it is then said to be **peronocarpous**. The second sub-order of the *Pyrenomycetes* exhibits this kind of fruit, and is called *Sphæriaceæ*.

Scheme of subdivision of the *Ascomycetes* Class:

Ascomycetes (Mycomycetes with endogenous spores)	(*a*) Asci naked			*Gymnoasceæ*.
	(*b*) Asci with envelope (Carpoascus) *Carpoasceæ*	(*a*) Carpoascus is a perithecium: *Pyrenomycetes*	1. Perithecium cleistocarpous	*Perisporiaceæ*.
			2. Perithecium peronocarpous	*Sphæriaceæ*.
		(*β*) Carpoascus is an apothecium		*Discomycetes*.

Of the *Discomycetes*, only a single species, *Sclerotinia Fuckeliana*, is of any importance in connection with the scope of the present work. The conidial fructification of this organism produces "sweet rot" in grapes, and on this account will be fully described in a separate chapter. Of the sub-order *Sphæriaceæ* we shall have three species to consider, namely: *Claviceps purpurea*, or the sclerotium of this organism, the detection of which in flour and bread is of interest to us; also *Sphærella Tulasnei*, and *Sphærella intermixta*. Of the sub-order *Perisporiaceæ* we shall have to make the acquaintance of a number of species belonging to the families *Penicillieæ* and *Aspergillieæ*. These *Carpoasceæ* will be dealt with in the penultimate section, wherein an example will be given of the structure and progressive development of the carpoascus.

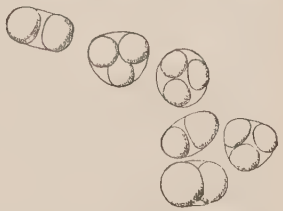


FIG. 122.—Carlsberg Bottom Yeast, No. 2.

Cells taken from a culture on wort gelatin, and exhibiting the formation of 2 to 3 ascospores in each. Magn. 1000. (*After Hansen.*)

Now the *Gymnoasceæ* are characterised by the nakedness of the asci, which remain devoid of integument during their entire existence. This sub-class (or order) can be divided into three families. In the *Saccharomycetes*, which constitute the lowest family and therefore the most simply constructed of all *Ascomycetes*, the mycelium itself is converted into an ascus, and there is here no separation into vegetative and fructificative organs. An example of this is given in Fig 122. A distinction between the organs is, however, exhibited in the other two families, the asci being articulated from the mycelium in one of them, namely, the *Exoasci*, where they form lateral offshoots resting on the mycelium. Finally, in the third

family, the *Gymnoasci*, which forms the connecting-link with the *Carpoasci*, incipient fructification is already found, as well as the intermediate organ, which we have already encountered under the name of ascogone.

We thus obtain the following scheme of subdivision of the Order *Gymnoasceæ*:

Order, or Sub-class.			Family.
Gymnoascece (asci naked)	(*a*) Without ascogones	(*a*) Mycelial cells themselves become asci	1. *Saccharomycetes.*
		(*β*) Special branches of the mycelium develop into asci	2. *Exoasci.*
	(*b*) Form ascogones		3. *Gymnoasci.*

Of these three families of *Gymnoasceæ* we are, in the present book, concerned with only one, namely the *Saccharomycetes*, the *Gymnoasci*, on the other hand, being of no importance so far as we are concerned, whilst the *Exoasci* may be dismissed in a few words. The genera of this last-named family have been divided by R. Sadebeck (IV.) into two groups: the one comprising parasites, the other saprophytes. The action of one representative of the former group, namely, *Taphrina Pruni* (also formerly known as *Exoascus Pruni*), will probably be familiar to the reader, viz., the malformation produced by this organism in green plums. According to Sadebeck (II.), the ascospores of this fungus, when grown in saccharine nutrient solutions, develop into a budding mycelium which excites weak alcoholic fermentation. A second species of this group, *Endomyces decipiens*, abounds in the lamellæ of the fruit of *Agaricus* (*Armillaria*) *mellens*, a parasite attacking timber and well known to foresters as "honey fungus." This second species of the *Exoasci* is worthy of mention here; in the first place because, as can be seen from Fig. 123, it forms an excellent example of a pleomorphous fungus; chiefly, however, because the ascospores are similar in shape to those of *Saccharomyces anomalus* (to be described later on) and its congeners. It was on the basis of this similarity that, some years ago, and again more recently, more than one mycologist was led to discuss the possibility of the *Saccharomycetes* having

Fig. 123.—Endomyces decipiens, Reess.

1. A mycelial branch, the lower part of which has developed three asci (*a*), each of them producing four hat-shaped ascospores; whereas the upper portion has separated into oidia (*b*). Magn. 320.
2. A fragment of mycelium entirely converted into oidia. Magn. 120.
3. A mycelial branch, which has formed oidia (*b*) at the top, but underneath has developed three chlamydospores (*c*). Magn. 240.
4. A mycelial branch with asci (*a*) solely. Magn. 320.
5. Pair of spores from such an ascus. Magn. 350. (*After Brefeld.*)

originated (§ 244) from this species or one nearly allied thereto. However, no one has yet succeeded in arriving at anything beyond mere hypotheses in this connection; and, moreover, similarly shaped spores are also found in other low *Ascomycetes*, e.g., *Ascoidea rubescens*. These few remarks are all that it is necessary to make, from our point of view, with regard to the *Exoasci* and *Gymnoasci*. On the other hand, all the following paragraphs, up to the penultimate section, will be concerned exclusively with the family *Saccharomycetes*.

FIG. 124.—Johannisberg Wine Yeast, No. 1. Young culture in wine must. Magn. 800. (*After Aderhold.*)

This family comprises three genera: *Monospora*, *Saccharomyces* and *Schizosaccharomyces*. As the name implies, the first genus is characterised by the fact that each ascus contains only a single spore. Up to the present only one species of this genus is known, namely, *Monospora cuspidata* (discovered by Metschnikoff), which is parasitic on and fatal to *Daphnidæ*: it is, however, beyond our province. Henceforward we have merely to deal with the other two genera, *Saccharomyces* and *Schizosaccharomyces*. Of the latter, only a few species are as yet known. Some of these are, nevertheless, worthy of attention, either from their technical importance, or from a physiological standpoint, and will therefore be dealt with in a separate chapter as soon

FIG. 125.—Schizosaccharomyces octosporus. One-day-old culture in beer wort at 25° C. Magn. 1000. (*After Schiönning.*)

as our main task is accomplished, namely, the study of the most important of the numerous species belonging to the genus *Saccharomyces*. In these latter the reproduction of the cells is effected by vegetative means; and under normal conditions almost exclusively by the process of budding described in § 219. An example is shown in Fig. 124. In the *Schizosaccharomyces*, on the other hand—as will be more fully elucidated later on—this process retires into the background and gives place to another, which closely resembles the reproduction of bacteria by fission, as was described in § 42. In this case, instead of putting forth, like

Saccharomyces, a bud which grows to the same shape and size as the mother-cell from which it sooner or later becomes detached, the cell develops a transverse partition wall, which then divides into two layers; and as soon as this is effected there is nothing to hinder the separation of the resulting daughter-cells (*see* Fig. 125). It is owing to this behaviour that the genus received the name *Schizosaccharomyces*.

The substance of the foregoing remarks can be employed for drawing up a scheme for subdividing the *Saccharomycetes* family:

			Genus.
Saccharomycetes (*Ascomycetes* with naked asci, the mycelium itself acting as an ascus)	(*a*) The ascus develops only a single spore		1. *Monospora*.
	(*b*) Ascus polysporous	(α) Vegetative reproduction by budding	2. *Saccharomyces*.
		(β) Vegetative reproduction by fission	3. *Schizosaccharomyces*.

Before entering upon the morphology and progress of development of the genus *Saccharomyces*, we will first occupy ourselves, in the following paragraphs, with the question of the alleged descent of the *Saccharomycetes* from other fungi; at the same time the opportunity will be utilised for defining the limitations and mutual relation of the terms *Saccharomyces*, budding fungi, and yeast.

§ 244.—The Question of the Origin of the Saccharomycetes.

For some years after they had been recognised as living organisms (§ 12) the cells forming the chief constituent of beer yeast, wine yeast, and pressed yeast, had to be content with a position in a corner of the botanical system. Occurring as a rule in the unicellular form, or at most in aggregations of uniform members, they offered little inducement for the attention of the systematist. Meyen and Schwann bestowed on them the new generic name *Saccharomyces* (§ 13), and relegated them to the fungoid kingdom. On the other hand, Kützing located them among the algæ, under the name *Cryptococcus*, for the very cogent reason that he failed to discover in them the formation of hyphæ, which is characteristic of the fungi. Although the views of the two first-named observers afterwards prevailed, and consequently the yeasts were recognised as belonging to the fungi, it was at first difficult to find suitable accommodation for them within the limits of this kingdom, no allied species being known with which they could be associated. In this emergency these and a few other similar fungi were classed together in a new group, namely, that of the *Blastomycetes* (budding fungi), which was interpolated as an intermediate link between the sub-kingdom of the fission fungi on the one hand and that of all the other fungi on the other. The desire, which then soon arose, to find a connection between this outside group and an order of the sub-kingdom *Eumycetes*, was therefore not an artificial one, but in harmony with existing circumstances and fully justified. The manner, however, of effecting the gratification of this desire, and also the unscientific standpoint adopted as the basis of procedure, cannot be termed praiseworthy.

From the explanation given in the introduction to vol. i. the reader is aware that the discovery of yeast as a living organism was coincident with a period when the dispute about primary origin raged very briskly. Now the yeast cell was one of the favourite objects of the attempt to prove the origin of organised from unorganised bodies. When, thanks to the labours of Pasteur and others, the theory in question was finally driven out of the realm of the exact sciences, many of its adherents considered an unconditional surrender was too much to be expected of them; and in order to preserve a little of the conception they assumed that, even if the parentless origin of the yeast cell from lifeless

material could no longer be claimed with certainty, there was a probability of yeast being evolved *from* lower fungi on the one hand, and *into* higher fungi on the other.

Two circumstances favoured the inception of this opinion and prolonged its existence. One of them consisted in the lack of positively reliable pure-culture processes for enabling the observer to start with a single individual (in this case a single cell), and trace its development with uninterrupted supervision, to the exclusion of all other germs. It would be setting too small a value on the circumspection of the workers in question and the adherents of the assumption that yeast is descended from other fungi, were one to suggest, in their justification, that they were not even dimly aware of the indispensable character of such a condition. They must therefore have recognised that their method of working was unreliable and led to deceptive results, and must have been inwardly convinced that the solution of this problem was the next task to be attempted. We have to reproach these experimenters that they did not follow this inevitable knowledge to its conclusion, and did not devote their whole energy to first elaborating a truly reliable method of working. This we must do because the confusing results they conjured up by the aid of their officious culture methods inflicted a twofold injury to science: first by aggravating the task of real research, and then by casting over the final results of the latter an anticipatory shadow of depreciation, which is solely due to these mycological necromancies.

The second circumstance favourable to the endeavours of those who tried to show the descent of yeasts from other fungi was the discovery of pleomorphism by Tulasne. Since the year 1851 this worker demonstrated, by a number of examples, that certain of the higher fungi were capable, under different conditions of nutrition, of assuming different forms; *e.g.*, at one time appearing as a mycelial thread and putting forth conidia, at another as a sclerotium from which proceeded small pileated fungi. With such an instance we shall become acquainted, in the case of *Sclerotinia Fuckeliana*, later on. In fact, in other words, it was thereby proved that many of the living forms which had hitherto been regarded as separate species of fungi merely formed a phase in the cycle of development of one and the same species of fungus. This theory of pleomorphism —which the inquiring reader will find fully described in the mycological handbooks already mentioned, more particularly in the treatise of A. Gilkinet (I.) recommended by A. de Bary—was established on an unassailable basis by its founder, by careful investigations of particular instances, and really marked a new epoch: not merely in mycology alone. On the other hand, it did a good deal of mischief at first in the study of yeasts.

The obvious objection that the lack of reliable methods of pure culture could also militate against the certainty of Tulasne's results can at once be disposed of by the fact that this authority worked with comparatively large fungi; and, moreover, a study of his works will very soon induce the conviction that such doubts are incapable of shaking the reliability of his discoveries. Furthermore, his determinations in connection with the progress of development—which were mainly based on microscopical researches—were afterwards confirmed by the cultures prepared by A. de Bary and others. The case was, however, different as soon as a crowd of imitators began attempts to prove the existence of similar pleomorphism in the less easily examined lower and lowest fungi. Whereas, in the case of the fungi examined by Tulasne—for instance, a sclerotium of *Claviceps purpurea*—it was easy to select an individual and test its development, it became necessary, with the microscopically small fungi, to start with a multiplicity of individuals, *e.g.*, a sample of yeast, owing to the absence of any method of pure culture. The detection of a small percentage of germs of other small fungi, *e.g.*, a few conidia of a mould fungus, was not such an easy matter, though the same

necessarily made their presence felt and even assumed a predominating position when the environment underwent a change in their favour. Either in ignorance of, or stubbornly misunderstanding, this state of affairs, Béchamp, in 1871, put forward the assumption that yeast cells could be developed from acetic bacteria. A year later, Trécul (II.) announced having made the same observation with spores of *Penicillium*. Conversely, according to Duval (I.), yeast cells were assumed capable of undergoing conversion into lactic acid bacteria. More extensive fallacies were advanced in 1875 by Robin (I.), giving renewed vitality to the reports of Pouchet (I.) and Bail, according to whom yeast cells are able to change to *Mucor*, *Penicillium*, *Aspergillus*, &c. Greater success was attained by H. Hoffman (III.), who even obtained a prize from the Paris Academy in 1870 for his conversions. For "staying power," however, the palm must be awarded to H. Hallier (II.), who still continued to uphold, in 1896, the hypothesis which had been relegated by A. de Bary to the "chronique scandaleuse" of science more than fifteen years earlier.

One extenuating circumstance to be considered in passing judgment on most of the above-named is the fluctuations of meaning sustained by the term yeast in the course of time. A backward glance on this point is therefore necessary, even on this ground alone. Apart from the vague terminology which permitted (§ 13) every fermentative agent, even the fission fungi, to be called yeast, sufficient indefiniteness attached even to the limitation of this term to the ferments belonging to the *Eumycetes*. For some years after Schwann's discovery the sole botanical definition of yeast was: a unicellular organism, reproducing by means of a peculiar method (termed budding), and exhibiting the power of forming alcohol and carbon dioxide from sugar. In 1857, Th. Bail (I.) discovered the occurrence of budding mycelia (§ 219) in the family *Mucoraceæ*, and ten years later assumed the descent of beer yeast from *Mucor racemosus*. Doubt on this point seemed to him to be the more inadmissible as he had noticed a weak alcoholic fermentation on submerging these budding *Mucor* mycelia in nutrient solutions containing sugar. Under the prevailing limitation of the term yeast, the name *Mucor* yeast was justifiable, though the same cannot be claimed for the generalised assumption, deduced from this observation, that a connection exists between the life-history of yeasts and that of the higher fungi.

Soon afterwards a successful endeavour was made by M. Reess (I.) to become better acquainted with the yeasts, before disputing over their relationship to other fungi. Following up the previous discovery of the formation of ascospores (§ 247) in the cells of wine yeast and beer yeast, he recognised the importance of this phenomenon for the systematology of the yeasts, and did it justice by announcing this peculiarity as a principal characteristic of the now remodelled genus *Saccharomyces*, which he relegated to the *Eumycetes* group. Subsequently the establishment of this characteristic led to a separation, which was objectionable from the standpoint of fermentation physiology: of the group of alcohol-producing budding fungi hitherto united under the common denomination "yeast," all those recognised as incapable of developing ascospores were rejected, and mostly, under the title "*non-Saccharomyces* of unknown systematic position," relegated to the group of *Fungi imperfecti*. The terms yeast and *Saccharomyces* ceased to be coincident. Many budding fungi capable of exciting alcoholic fermentation, *e.g.*, several of the genus *Torula*, which still have some claim to be considered as yeasts, are therefore excluded from the *Saccharomyces* family because of their inability to put forth ascospores. On the other hand, there are certain true *Saccharomycetes* which are unable to excite alcoholic fermentation, and therefore have no title to the name yeast (in the above sense of the term), *Saccharomyces membranæfaciens* being an example.

After the publication of Schwann's discovery, that of Reess was the first advance in the systematology of the yeast fungi; and it was left to E Chr. Hansen to make the next move, viz., to separate the units (species, races, varieties) of the genus *Saccharomyces*, introduce experimental investigation into systematic description, and, for the first time, base this research on really pure cultures. This he succeeded in accomplishing by degrees, so that the *Saccharomycetes* now form a large and well-defined family.

Of the objections laid against the determinations made by Reess, that put forward by Brefeld (V.) must be briefly considered. This worker observed, and reported in 1883, that the spores of smut fungi (*Ustilagineæ*, *q.v.*), when grown under special conditions, bud like yeast (Fig. 126), and that the process can be caused to repeat itself as often as desired. These yeast conidia (p. 16), as they were termed by Brefeld and others, are incapable of exciting alcoholic fermentation or producing ascospores. With regard to this deficiency, it was justly remarked by Reess (II.), that while Brefeld's observations increased the number of instances of yeast-like budding in the higher fungi, they by no means disproved the former worker's demonstration of the independence of the genus *Saccharomyces* and its allocation to the *Ascomycetes* group. Eight years later, Brefeld (IX.) repeated his hypothesis that the yeasts must be regarded as conidia from higher fungi (of a still unknown genus). He was reminded by E. Chr. Hansen (XVIII.) that the accuracy of this assertion still remained to be proved; and this latter worker also demonstrated, from existing data, that no connection between the life-history of the *Saccharomycetes* and that of any other fungi could possibly have been proved hitherto. Similar expressions of opinion have been uttered by A. de Bary, Zopf, H. Will, and others.

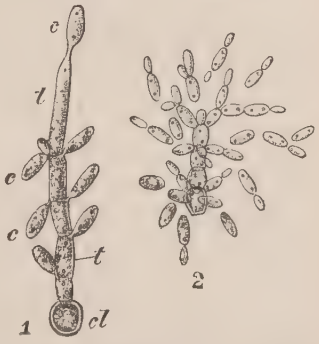


Fig. 126.—Ustilago carbo, the cause of smut in oats.

1. The spore *cl*, grown in a nutrient solution, has produced a polycellular mycelium (*t*), which has put forth yeast-like conidia (*c*). Magn. 450.
2. Chains of buds from these conidia. Magn. 200. (*After Brefeld.*)

As already stated on several occasions (§§ 220 and 243), the ascus can be distinguished from the sporangium by its more definite form and by the number, shape, and method of formation of its spores. This precision of form must be present in any fungus before the latter can be classified with the *Ascomycetes*. It is most decided in the more highly developed species, and becomes progressively less so as we descend the systematic scale; and, as a matter of fact, it is low (especially as regards the number of spores produced by the ascus) in the *Saccharomycetes*, which are the lowest of the *Ascomycetes*. In this genus, even in one and the same species, the number of spores is not always the same in each ascus, and not infrequently an odd number is present. A similar lack of precision is also exhibited in other genera, such as *Ascoidea*, *Protomyces*, and *Thelebolus*. Brefeld (IX.) would associate all these into a special group, to which he gave the name *Hemiasci*, intending thereby to express the imperfect character of their asci, and that the development of the latter from the sporangia had been, as it were, arrested half-way. Therefore, according to his ideas, the *Hemiasci* formed a connecting-link between the sporangiogenic *Phycomycetes* and the true *Ascomycetes*. Brefeld then endeavoured to effect an analogous separation between the other *Mycomycetes*, which, as we are aware, differ from the *Ascomycetes* by lacking the capacity for producing endogenous spores, and fructify by means of conidia. In the highest of these, namely, the *Basidiomycetes*,

the conidiophores is developed into a basidium (§ 384) On the other hand, in a number of *Mycomycetes* that do not exhibit ascofructification (viz., the *Ustilagineæ* and the *Tilletieæ*), the precision of the conidiophores is not so great as in the rest. Consequently they were separated—under the name *Hemibasidii*—from the latter, or *Basidiomycetes;* and, according to Brefeld's ideas, they formed an intermediate link between the *Phycomycetes* group (with conidial fructification) and the *Basidiomycetes*. They therefore constituted a branch of the *Hemiasci;* and these latter were grouped by Brefeld along with the *Hemibasidii*, to form an intermediate kingdom, under the new name of *Mesomycetes*. This conception, which is more fully detailed in a work by F. von Tavel, and according to which the *Saccharomycetes* should no longer be regarded as full-fledged *Ascomycetes*, was opposed by W. Zopf; but the final settlement of this highly complex question has not yet been reached. For us the *Saccharomycetes* will still continue to rank with the *Ascomycetes*, sub-class (order) *Gymnoasceæ*, and not as *Hemiasci*.

Any worker who attempts to trace a relationship between the *Saccharomycetes* and other fungi must agree with Reess's classification of the former with the *Ascomycetes*. Nevertheless, there is still room for diversity of opinion within the above limitation of the question. Thus, A. de Bary had already shown the great agreement, in structure and other particulars, between the *Saccharomycetes* and certain of the *Exoasceæ*, and remarked that the former might at once be ascribed to this latter group. Another matter worthy of attention is the question regarding any eventual connection between the *Saccharomycetes* and certain *Hyphomycetes* which have hitherto been included in the group of *Fungi imperfecti*. Some warrant may be accorded to such enterprise, though not without attention being drawn at the same time to the circumstance that a successful result must primarily be placed to the credit of these *Hyphomycetes*, since they would thereby obtain recognition as forming part of the cycle of development of an *Ascomyces*, and consequently take a step upwards in the botanical system. This point, which may also lay some claim to the attention of earnest investigators, will be briefly touched upon in a later chapter dealing with *Dematium pullulans*. At present, on the other hand, we have to deal concisely with a couple of recent assumptions, or rather with the demonstration of their inapplicability.

In 1895 J. Juhler (I.) enlightened the world with the sensational report that he had succeeded in so influencing a species of *Aspergillus* as to cause it to develop *Saccharomyces* cells that produced alcohol. A communication shortly afterwards issued by Alfred Jörgensen (II.), in whose laboratory the alleged discovery was made, explained that *Aspergillus oryzæ* was in question. They were quickly followed by E. Sorel (I.), who completed the beautiful circle by an alleged case of retrogression to the original mould fungus on the part of yeast cells stated to have originated from this *Aspergillus*. It was disturbing to observe the amount of perplexity induced by these publications, not among mycologists but on the part of a large number of fermentation technologists, as can be seen, for instance, in the case of a treatise by Eckenroth and Heimann (I.), who occupied themselves in a very similar manner with a *Penicillium*. In the interest of this branch it was necessary and indispensable that these hypotheses should be examined and refuted in all points by a number of workers schooled in botany. The credit of having shared in this task—a somewhat thankless one from the scientific standpoint—is chiefly due to Klöcker and Schiœnning (I. and II.), and also to O. Seiter (I.), C. Wehmer (VIII.), and J. Wortmann (IX.). All of them arrived at the harmonious conclusion that not under any circumstances, even the conditions of culture selected by Juhler, Jörgensen, and Sorel, could any development of yeast cells take place from *Aspergillus oryzæ*, or conversion of *Saccharomyces* cells into this mould fungus.

The same also applies to other species of *Aspergillus* examined with the same object by KLÖCKER and SCHIÖNNING.

Consequently it may be averred that no proof, in any particular, has yet been afforded in support of the hypothesis that *Saccharomycetes* are derived from other fungi.

We therefore maintain that the distinguishing characteristic of the term *Saccharomyces* is a purely botanico-morphological one, namely, the capacity of the cell for producing ascospores. The method of formation of the latter will be fully described in § 247. All the *Saccharomycetes* are budding fungi, *i.e.*, they vegetate in the form of a budding mycelium of the kind described in § 219. On the other hand, however, not every budding fungus is a *Saccharomyces*. The term yeast has two important characteristics: one morphological, the other physiological. In the strictest sense the term is applied to such budding fungi as are capable of exciting alcoholic fermentation, and therefore the alcohol-producing Mucors are not to be classed as yeast; neither are the *Saccharomycetes* that lack fermentative power. Even in PASTEUR'S (III.) "Studies on Beer" no mutual limitation is placed on the terms *Saccharomyces* and yeast, but both are regarded as interchangeable, so that in many places we are unable to ascertain which is meant. The chief interest attaching to the yeasts is in respect of their practical utility. In many treatises on the physiology of the ferments the question whether they should be considered as *Saccharomycetes*, or as budding fungi of some other species, is left untouched. It is therefore impracticable to relegate the species there mentioned to one or the other group; and in such cases there remains no alternative but to speak generally of "yeast." This has also been done, and should be so understood, in the following paragraphs.

In laboratories where pure yeasts are cultivated for the purposes of the practical fermentation industry, it is very seldom that the rules of scientific nomenclature are adopted by supplementing the term *Saccharomyces* with a specific name, even when the species of the organism is known with certainty, the usual practice being to name the yeast in accordance with the locality of origin. Thus the Johannisberg wine yeast No. 1, illustrated in Fig. 124, was obtained from the sediment of a young Johannisberg wine. "Saaz yeast" is a bottom-fermentation beer yeast isolated by P. Lindner from the stock yeast of the Saaz brewery (Bohemia). This yeast will be frequently mentioned later, on account of its very low power of attenuation. The very high-attenuation "Frohberg yeast" originated at Frohberg's brewery, Grimma (Saxony). In many instances the yeast is simply given a number, under which it is registered in the laboratory collection (living herbarium) and is cultivated further. Thus, the distillery yeast known to distillery technologists and mycologists under the abbreviated title "Race II." is the second of a series of yeasts given out by the Berlin Experimental Station for practical testing in respect of their utility, the species in question finally proving superior to the others.

It may be remarked casually that a proper discrimination between the three expressions, budding fungus, *Saccharomyces*, and yeast, is often lacking in medical treatises dealing with a pathogenic budding fungus. Some of these are known to produce illness, even attended with fatal results, when they find the conditions of development favourable in the body in which they have made their habitat. The literature on this matter has been collected by J. RAUM (I.), and in a monograph by O. BUSSE (I.), as also in the different volumes of P. Baumgarten's *Jahresbericht*. These species are oftentimes spoken of as *Saccharomyces*, or yeast, although their connection with this genus, or their faculty of exciting fermentation, is more or less doubtful. This remark applies to the so-called *Sacch. farciminosus Tokishiye*, *Sacch. neoformans*, and *Sacch. lithogenes Sanfelice*; *Sacch. sphæricus* and *Sacch. ovalis van Hoorn*, &c. One species recognised as exciting

fermentation, and consequently to be classed as a yeast, though not as yet proved to be a true *Saccharomyces*, is a budding fungus discovered by O. Busse, and observed by him to produce, in the human body, a kind of general debility (*Saccharomycosis*) which may terminate fatally. That certain true yeasts are pathogenic when artificially introduced into the arterial circulation was proved in 1892 by Hueppe, in the course of experiments with Rauenthal wine yeast and young porpoises. This result has been repeatedly confirmed since, notably by L. Rabinowitsch, in a series of experiments with about fifty stocks of different species and origin, seven of which proved pathogenic.

§ 245.—Bottom Yeasts.

If a number of flasks be charged with a clear nutrient solution, of a kind favourable to the growth of yeast and containing a fermentable sugar, and each of them be inoculated with a trace of a pure culture of different yeasts, such as are used in brewing, distillery work, vinification, &c., the cultures being then kept at room temperature for a couple of days, it will be found that cell reproduction and fermentation—manifested by the appearance of turbidity and gas bubbles—will occur in all. It will thereafter soon be possible to separate the flasks into two groups, according to the appearance presented. In the one group the yeast crop developed from the sowing will remain almost entirely within the liquid throughout the entire period of fermentation, and mostly at the bottom even from the start. Yeasts of this kind are termed **bottom yeasts**, and excite **bottom fermentation**, the yeast crop being sedimental.

In the other group the fermentation is very brisk and attended with the formation of large quantities of froth (head); and in the earlier stages a larger or smaller number of the new cells are raised to the surface by the ascending bubbles of gas, and remain there—provided the vessel be high enough to prevent frothing over—until fermentation is terminated and the froth breaks up, whereupon they sink down to the bottom of the liquid and increase the sedimental deposit. This kind of fermentation is termed **top fermentation**, and the yeasts producing it are called **top-fermentation yeasts.** Typical examples of bottom-fermentation yeasts are afforded by the Munich lager-beer yeasts. On the other hand, the most highly developed top-fermentation yeasts are the species forming the bulk of the pressed yeast prepared by the old (Viennese) process. This pressed yeast consists exclusively of cells raised from the mash by the head, and of the daughter-cells of same, since it is impracticable to separate from the mash such cells as are left therein. These two extreme types of yeast are connected by a number of intermediate grades. The question of another, and fundamental, difference between top- and bottom-fermentation yeasts will be discussed in the paragraphs relating to melibiase.

As soon as the primary fermentation is manifestly at an end, let us take a trace of the sedimental yeast from each of the flasks, thin each sample down with a drop of water on a glass slide, cover it with a cover-glass, and examine it under a high power (250–500) in the microscope. In many of the specimens the cells will be found globular or oval in shape; speaking generally, most of the beer yeasts and brandy yeasts will present this appearance. Now because, since the time of Meyen, the name *Saccharomyces cerevisiæ* has been usually applied to beer yeast, it has gradually become the custom to say of yeasts exhibiting approximately globular or oval cells of large dimensions that they are of the **cerevisiæ type**. Fig. 127 gives an example of this class, namely, a top-fermentation yeast isolated by Hansen (XII.) from the stock yeast (of which it formed the main constituent) of a top-fermentation brewery in Edinburgh and called by him *Sacch. cerevisiæ I.*

The sedimental yeast in other flasks will be found to differ from the foregoing, inasmuch as it contains cells which, instead of being globular or oval, are elliptical in shape. Yeasts of this kind were invariably found in fermenting must by REESS (I.), who called them *Sacch. ellipsoideus*, which specific name

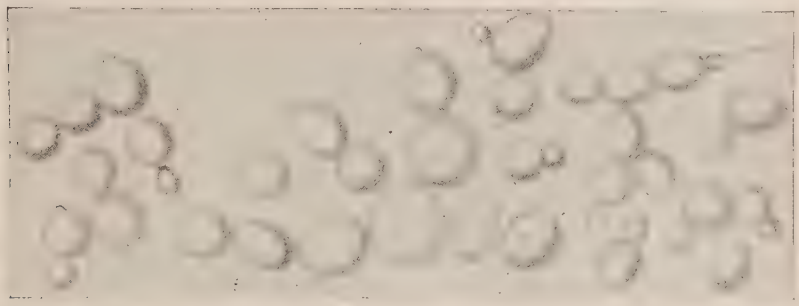


FIG. 127.—Saccharomyces cerevisiæ I., Hansen.
Cells from the sediment of a young culture in beer wort. Magn. 1000. (*After Hansen.*)

gradually became enlarged to a morphological designation, so that we therefore speak of this or that yeast as being of the **ellipsoideus type**, meaning thereby solely, in the first place, that the cells of such species are generally elliptical in shape and rather smaller than those of the cerevisiæ type. Fig. 128 gives an

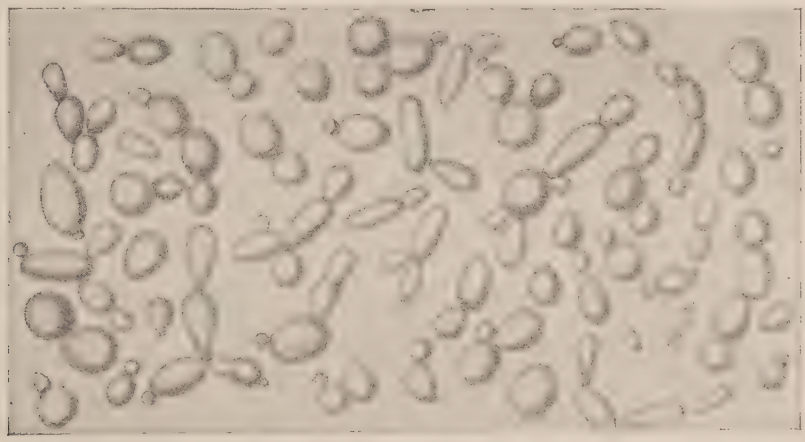


FIG. 128.—Sacch. ellipsoideus I., Hansen.
Cells from the sedimental yeast of a young culture in beer wort. Magn. 1000. (*After Hansen.*)

example in the *Sacch ellipsoideus I.* obtained by HANSEN (XII.) from the surface of ripe grapes. This type is exhibited by many species of wine yeast; and it is therefore easy to understand why, in the absence of a method of pure culture and the consequent impossibility of determining the existence of divers species, Reess's appellation of *Sacch. ellipsoideus* very soon became a synonym for wine yeasts in general. This custom is, however, now no longer justifiable, since we are nowadays acquainted with races of wine yeasts whose cells cannot be classed with the ellipsoid type, but are globular or elongated. Conversely, not

every yeast of the ellipsoideus type is a wine yeast. An example of such a divergent yeast—though in respect of the shape of the cells in the yeast sediment it is almost identical therewith—was isolated by HANSEN (XII.) in 1883, from the stock yeast of the Tuborg brewery in Copenhagen, namely, the so-called *Sacch. ellipsoideus II.* This yeast occurred there, in association with *Sacch. Past. III.*, as a technically pathogenic yeast, inasmuch as it produced disturbances in the brewing, or rather tended to form haze in the trade casks or bottles.

The third group, consisting of the remainder of the flasks used in our experiment, differs from the other two, inasmuch as the cells of the young sedimental yeast are of elongated form, something the shape of a sausage or a short tube with closed ends, and in some species slightly constricted in one or two places. Cells of this kind were noticed by PASTEUR (XII.) during his researches on wine. They were also found by REESS (I.) in the secondary fermentation of certain wines examined by him; and this worker named them *Sacch. Pastorianus* in honour of the French scientist. Cells of this type have been more frequently observed by subsequent investigators, and this specific name has been gradually modified into a morphological term. When it is said of a yeast that it exhibits pastorianus forms, or is of the **Pastorianus type**, the term merely implies that under normal conditions the sedimental yeast of the species in question chiefly forms cells that are sausage-shaped, and not globular, oval, or elliptical. An example of this kind is afforded by *Sacch. Pastorianus I.*, illustrated in Fig. 129. This species was discovered by HANSEN (II. and XII.), in 1880 and 1881, in the air at the Alt-Carlsberg brewery, Copenhagen, and was introduced into the literature under the above name, by him, in 1882, after he had succeeded in proving that it had also crept into the stock yeast of this brewery, imparting to the resulting beer an obnoxious bitter by-flavour and a smoky smell. It is therefore a virulent **pathogenic yeast** (in the technical sense).

It would be a great mistake on the part of the reader to assume, from the foregoing sketch of the three main typical forms of yeast cells, that each species of yeast invariably assumes the same form. This is not the case, a powerful influence on the form being exerted by the conditions of cultivation. This last fact was unknown, and indeed undiscoverable by the methods in use, at the time Reess set up his specific classification based solely on the form of the cells. Thus, up to the year 1882, it was thought that the bottom-fermentation yeast used in brewing always consisted of the one single species *Sacch. cerevisiæ;* and it was not until 1883 that E. CHR. HANSEN (XII.) showed that we have to reckon with a large number of species, and that consequently the names *Sacch. cerevisiæ*, *Sacch. Pastorianus*, *Sacch. ellipsoideus*, &c., could henceforth be merely used as group names. Since that time, no small portion of this worker's investigations has been devoted to the question of the dependence of cell form on the conditions of cultivation, and to the elucidation of the fact that, morphologically, the character of a species of yeast does not reside in the form of the cell alone, but also in the manner of its dependence on the external conditions of which it is the result. If these conditions be known to a certain extent, then the form of the cells constitutes a very valuable and fairly reliable indication. Since, like other manifestations of vitality, the form of the cells is a resultant of two components, namely, inherited properties and the sum of all the external influences, it is evident that, even if the uniformity of the latter conditions could be made absolutely certain, the former reason alone would preclude the expectancy of perfect regularity in the cells of a culture. Moreover, owing to the imperfect state of our knowledge, both in chemistry and physics, the production of absolutely identical conditions of existence in two cultures started at different times is unattainable. Again, even when working with a single cell, it will be found that the daughter-cells, grown in one and the same nutrient medium, differ

among themselves; in one of them one of the inherited properties latent in the mother-cell makes its appearance, whilst in another of the cells other inherited properties preponderate. It is necessary to lay emphasis on this point, because it only too often happens that a beginner in the cultivation of yeast feels compelled to lose all faith in his knowledge when he observes that a pure culture, prepared from a single cell strictly according to all the rules of the method, consists of cells all more or less different in size and form. Now there is nothing special about such variability, which is exhibited by bacteria and all other living organisms, and that too in no less a degree than with the yeasts. Nevertheless, within the limits afforded by the aforesaid difficulty of control, one and the same

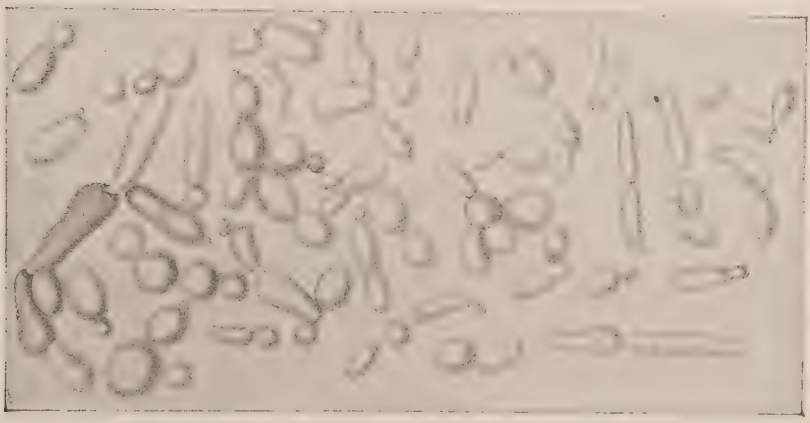


FIG. 129.—Saccb. Pastorianus I., Hansen.

Cells from the sedimental yeast of a young culture in beer wort. Magn. 1000. (*After Hansen.*)

species grown under the same conditions will give cells of approximately uniform shape, say, for instance, that of the sedimental yeast of the cerevisiæ type.

That under these conditions it is actually possible to trace differential characteristics may be explained by the aid of the two drawings shown below, both of them representing samples from the sedimental yeast of a culture in beer wort, at the close of the primary fermentation. Fig. 130 is the so-called Carlsberg Bottom Yeast No. 1, the first yeast prepared by the pure-culture method, and by means of which E. Chr. Hansen introduced his pure-culture method into practice, at the Alt-Carlsberg brewery, in 1883. It was isolated as the principal constituent of the same stock yeast, which was found to be infected with the aforesaid *Sacch. Pastorianus I.* A characteristic feature of this yeast is the preponderance of pointed oval cells, those of purely globular form being very much in the minority. Elongated cells also are very rare. On the other hand, the Carlsberg Bottom Yeast No. 2, shown in Fig. 131, is characterised by the more rounded appearance of its cells, and by the occurrence of unusually large, or giant, cells, one of which can be seen on the left of the illustration. The appearance of these giant cells is specially remarkable in some species, and then forms a good indication. Thus, BEYERINCK (XX.) found large cells attaining as much as 20 μ in diameter, in old agar-agar cultures of a budding fungus known as *Sacch. Kefyr*, which he had isolated from the Kephir to be described in our final chapter, whereas the other cells measured only 5 to 6 μ.

The **sedimental yeast** found deposited at the bottom of the fermenting vessels at the close of primary fermentation, in bottom-fermentation breweries,

is drawn off—after the removal of the young beer—through an orifice in the bottom of the tun, and is collected in a vessel wherein it is washed with water, to be afterwards stored under ice water until required for pitching a subsequent brew. This yeast, it may be stated, is a highly diversified mixture. Apart from the possible presence of several species of yeast and bacteria (*sarcina*, &c.), it also contains sundry other ingredients, the most important of which are: First, salts of lime, chiefly the oxalate, immediately recognisable under the microscope by its octahedral, rhombohedral or flat tabular crystals. These have been precipitated during fermentation. Their origin is only to a small extent attributable to the metabolism of the yeast; they also occur in unfermented wort, and in

FIG. 130.—Carlsberg Bottom Yeast No. 1.

Cells from the sedimental yeast at the close of primary fermentation. Magn. 1000. (*After Hansen*).

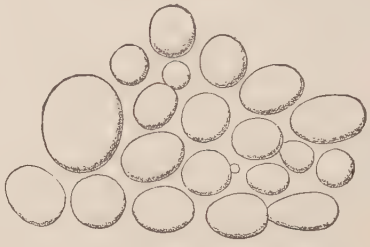


FIG. 131.—Carlsberg Bottom Yeast No. 2.

Cells from the sedimental yeast at the close of primary fermentation. Magn. 1000. (*After Hansen.*)

wort gelatin, and, it may be remarked in passing, disturb the beginner when he examines such a gelatin plate under a low power (30 to 100) before inoculating with micro organisms, or employing it for starting a single-cell culture. Secondly, the yeast mixture contains precipitated hop resins, in the shape of very small globules, sometimes united as aggregations; they give the resin reactions, and therefore assume a handsome red coloration in presence of alcoholic tincture of alkanna root. Thirdly, there are the so-called glutin bodies, which are fine globules of an albuminous nature, originating in the malt and precipitated from the wort at the low temperature of the fermenting cellar. They have formed the subject of some deep researches by H. WILL (III.). Fourthly, certain dark brown fragments, which are mostly looked on as hop resins by practical brewers, but in reality are said by H. WILL (IV.) to give all the reactions for albumen. When present in large quantity they form a source of trouble in brewing, by enveloping the yeast cells and rendering these latter inoperative. The upper layer of the sedimental yeast in the vat containing the beer in condition for racking is specially rich in such extraneous admixtures, and is consequently rather dark in colour. This portion is generally removed in advance and thrown away, before the underlying "white" yeast is drawn off. Fifthly come mucilaginous matters of different kinds (§§ 254 and 255), which have been excreted by or extracted from the yeast cells. Sixthly are residual fragments of the mashed materials, hops, lupulin granules, and not infrequently aphides, and the like.

It has already been stated that all the samples examined were taken from fresh cultures, *i.e.*, cultures in which the primary fermentation was just terminated and the deposited yeast crop was of recent date. On the other hand, a different appearance is presented by the cells of a sediment that has lain for some time under the fermented liquid—that is to say, in old laboratory cultures, or the sludge found at the bottom of lager-beer storage vats, and therefore consisting of cells that have been exposed to the influence of the supernatant beer for some considerable time (often several months). Under these circumstances a

large number of elongated cells of the *Pastorianus* type are produced, even in yeasts that are ordinarily of decidedly cerevisiæ type. This is a very useful thing for the beginner to know, in order that he may not be disheartened on finding a number of cells of the suspicious *Pastorianus* type among the sludge of a vat, the beer in which was fermented with a pure-culture yeast. Provided the brewing operations have been conducted in a cleanly manner, the process of spore analysis, described in § 247, will quickly reassure him of the absence of infection.

Up to the present the composition of the **deposit in wine** and the "wine yeast," in the sense used by tartaric acid manufacturers, has not been so thoroughly examined as is the case with the sedimental yeast of breweries. Next to yeast, the chief constituent is cream of tartar, which is gradually precipitated in the crystalline form by the increasing amount of alcohol in the fermenting wine.

When exposed to very unfavourable conditions, the yeast cell assumes a true involution form. Cells of this kind, resembling amœbæ in contour, are found, for example, in very old gypsum-plate cultures, or similar cultures on wort gelatin. They have been drawn by P. Lindner (VI. and X.). Many cells that have become exposed to unfavourable conditions in sedimental yeast are able to withstand the same and survive uninjured, because they have previously laid up a considerable store of material and greatly strengthened their membrane. Under these circumstances they become permanent cells, which may be regarded as gemmæ (§ 223). These forms will be further discussed in §§ 246, 249, and 251.

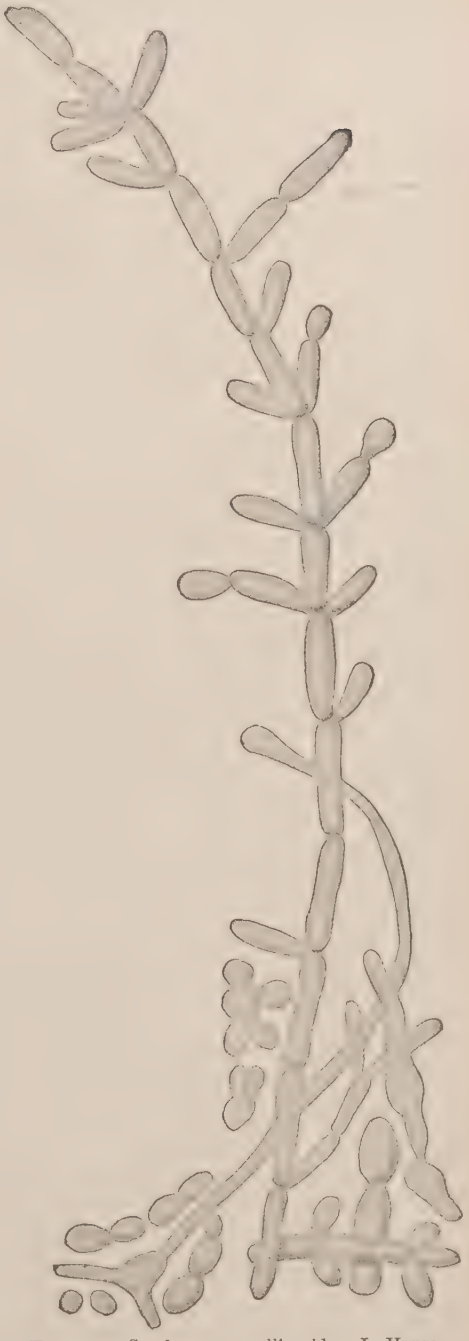


Fig. 132.—Saccharomyces ellipsoideus I., Hansen. Cells and chains of cells from the film of an old culture in beer wort. Magn. 1000. (*After Hansen.*)

§ 246.—Film Formation.

Yeast cultures, started in the manner foreshadowed at the com-

mencement of the preceding paragraph, are able to exhibit another form of development. If the samples have been taken with every care, all infection being prevented, and the vessels again closed with their germ-proof plugs of cotton-wool—which, however, do not exclude air—all that is then necessary is to store them in a quiet corner of the laboratory for some time (several weeks or even months), to find that **film** has developed on the surface of the liquid. The structure and physiological behaviour of this film will now be described.

This form of development originates in cells, which, thanks either to the presence of some fatty excretion adhering to the outside of the membrane, or to some albuminous or resinous deposit acquired from the nutrient medium, are able to float on the surface of the liquid when the latter becomes quiescent after

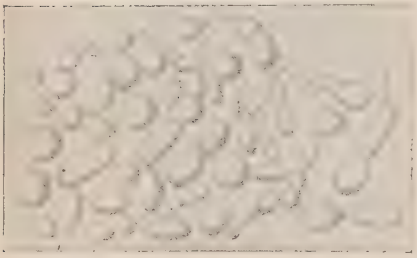


FIG. 133.—Sacch. Past. II., Hansen.

FIG. 134.—Sacch. Past. III., Hansen.

Cells of film grown on beer wort at 20° to 28° C. Magn. 1000. (*After Hansen.*)

fermentation has terminated. A good opportunity for these cells to remain afloat is afforded at the places where the surface of the liquid is in contact with the walls of the (round) culture vessel. Hence, it is in such positions that the development of the film will be earliest observed, in the form of a whitish ring. In many instances this ring is by no means complete at first, but appears in the form of a series of patches, *i.e.*, **colonies of cells**, which gradually increase and then meet, coalescing to a closed ring. In other cases the annular formation is less fully developed, and the growth of the film proceeds from small colonies or islets of yeast which have originated on the surface of the liquid itself. Other conditions being equal, the dimensions attained by the film vary according to the species of yeast.

The form of the cells from which the film is constructed differs in general from that of the sedimental cells, by attaining greater **longitudinal development** (up to 150 μ and over), whilst the transverse measurement is often less than in cells of sedimental yeast. The second characteristic is a more or less **abundant branching**. An example is shown in Fig. 132.

The time required for the development of the film to become manifest differs, other conditions being equal, with the species of the yeast, and is longer the lower the temperature of the culture. According to a series of determinations made on species of *Saccharomyces* by HANSEN (XVI.), the time required for *Saccharomyces cerevisiæ I.* at 33° to 34° C. is about 9 to 18 days; at 20° to 28° C., about 7 to 11 days; at 13° to 15° C., about 15 to 30 days; at 6° to 7° C., about 2 to 3 months. No formation of film occurred above 34° C., or below 5° C.

These limits of temperature, which also apply to all the other species

examined, are therefore more restricted than those wherein the yeast is able to bud and incite fermentation. Film formation is therefore precluded in the fermenting cellars of bottom-fermentation breweries, the temperature here being, wherever possible, maintained between 0° and 2° C. It has already been shown by Hansen, and confirmed by H. Will (VIII.), R. Aderhold (I.), and other workers, that the time elapsing before the film makes its appearance, and the dimensions attained by the film, are very greatly dependent on the conditions of cultivation (composition of the nutrient solution, and also in a high degree on the method of sterilisation, supply of air, &c.).

In some cases morphological peculiarities in the film cells afford a means for differentiating the species. With *Sacch. Past. II.* and *Sacch. Past. III.* this can

Fig. 135.—Sacch. Past. III., Hansen.

Cells in film grown on beer wort at 18° to 15° C. Magn. 1000. (*After Hansen.*)

be recognised in a beautiful manner, and at the same time a fresh instance is afforded of the dependence of cell form on temperature. The first-named, weak, harmless, top-fermentation species was isolated by Hansen from the air of the Carlsberg brewery. The other, which is of decidedly top-fermentation character, was obtained as a pure culture from a Copenhagen lager beer suffering from haze, and was recognised as the cause of the malady. It is difficult to distinguish between them by the form of the cells present in the sedimental yeast, both being very similar to *Sacch. Past. I.* (*see* Fig. 129). The same also applies to the component members of their films grown at 20° to 28° C. and illustrated in Figs. 133 and 134. The case is, however, different—as Hansen (XVI.) has shown—when the two have been allowed to form films at 13° to 15° C., since under these conditions one of them, *Sacch. Past. III.*, exhibits a number of highly elongated cells (Fig. 135) with lateral buds, whilst those of *Sacch. Past. II.*, on the other hand, retain very much the same form as when grown at 20° to 28° C., *i.e.*, only slightly elongated, and even globular. An essential precaution for this comparison is the use of young films, since when the films are very old the No. II. species exhibits elongated cells just like those of No. III. Similar conditions in this respect obtain between *Sacch. ellipsoideus I.* and *Sacch. ellipsoideus II.*, Hansen.

The faculty of developing a film on the surface of suitable nutrient solutions is shared by nearly all the budding fungi, both *Saccharomycetes* and *non-Saccharomycetes*. Of these latter we shall deal (in the last section), under the generic

name *Mycoderma*, with a special group, the species of which are widely distributed and grow spontaneously on the surface of wine or beer when the latter are exposed to the air, a quick-growing, wrinkled skin (mould film) being formed. Air being highly essential to *Mycoderma*, these organisms normally grow exclusively in the form of a superficial film on the nutrient solution. Owing to this peculiarity they are the cause of disturbance in researches into film-formation in the true yeasts, when the latter are not grown as pure cultures but contaminated with the very abundant *Mycoderma*. On this account objection may be raised against the reports of Reess (the first to observe the production of films by true *Saccharomycetes*), and also those of Pasteur (III.). In 1876 the latter characterised as aerobic or mould yeasts the film developing on the surface of fully fermented wort. He halted between two opinions with respect to this phenomenon, one being that it was a special (*i.e.*, aerobic) condition of development of the beer yeast residing at the bottom of the fermented liquid; whilst the other looked on the film as composed of extraneous cells undesirably present with the sowing. It was not until Hansen applied pure cultures to these investigations that a decision could be formed on this point.

Fig. 136.—Saccharomyces Past. II., Hansen. Cells in film grown on beer wort at 13° to 15° C. Magn. 1000. (*After Hansen.*)

The question of the convertibility of bottom-fermentation yeast into top-fermentation yeast, and *vice versâ*, is also touched by the foregoing explanations. Pasteur was of opinion that the "aerobic" cells constituting the film that had developed it at the close of primary fermentation in his cultures inoculated with (impure) bottom-fermentation yeast were capable of exciting top fermentation when transferred to a fresh nutrient solution. He even gave a recipe by means of which the brewer could prevent any such undesirable conversion of the stock yeast. This theoretically and practically important question was afterwards taken up by Hansen, who found that the descendants of the films of all the species of bottom yeasts examined by him in this connection invariably produced nothing but bottom-yeast cells when inoculated into fresh nutrient solutions, even when exposed to a temperature (26° C.) very favourable to the progress of top fermentation.

The true *Saccharomycetes* can be separated—though not very sharply—into two groups: one of which does not form films until the primary fermentation is terminated and the sedimental yeast has all come down; whilst in the other group growth proceeds on the surface from the commencement, and indeed in many cases exclusively so. An instance of this latter kind is afforded by the *Saccharomyces membranæfaciens*, first discovered by E. Chr. Hansen (VIII.) in the mucinous discharge (§ 248) from elm, and later in well-water by J. Köhler (I.).

The separate stages of the development of film were closely investigated in four species of bottom-fermentation yeast by H. Will (VIII.). At the outset no difference can be detected between the cells of the sedimental yeast and those retained floating on the surface by flakes of albumen and residual fragments of the "head," and from which the development of the yeast islets originates.

Later on, however, it is observed that these floating cells produce daughter-cells, the chief feature of which—as may be seen from a comparison of Figs. 137 and 138—is that, instead of appearing singly or in pairs on the mother-cell, as they do in the sedimental yeast, a number are formed simultaneously thereon. They are also much smaller (*e.g.*, only 7 μ as compared with 10 μ) than those in the sedimental yeast, are oval or sausage-shaped, and in turn produce similar daughter-cells, the whole remaining connected together. These cells form a new and special generation, which Will terms the **first generation of true film cells**, since with their appearance the formation of the film begins. In addition to

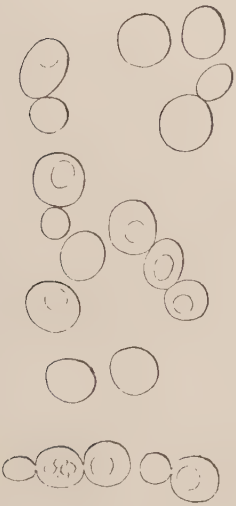


FIG. 137.—Cells of sedimental yeast from a wort culture of—

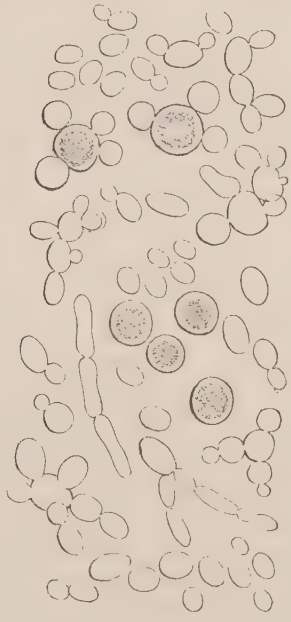


FIG. 138.—Film cells of the first generation, and permanent cells from the film of a wort culture of—

Bottom-fermentation beer yeast No. 93 of the Munich Brewing Station. Magn. 750. (*After Will.*)

these there quickly form other cells, which are characterised by considerably thicker membrane and an abundant content of glycogen and fat. These prove to be true permanent cells, both on account of their anatomical structure and their physiological behaviour, since they alone retain vitality and are capable of development when the cultures are old and all the other cells and the film have perished. The yeast ring, which grows about the same time, is specially rich in these permanent cells. They soon produce highly elongated, sausage-shaped or tubular cells, which in turn behave in a similar manner and also produce analogous lateral daughter-cells, the result being the formation of many-membered chains of the kind shown in Fig. 132. Will terms the members of these bands film cells of the second generation. The older the film the more luxuriant do they grow, and the farther do the film cells of the first generation retire to the background. At a later period, partition walls are formed—more or less abundantly in the different species—in these elongated cells; and similar septa are also found in the chains of buds resulting from the germination of the permanent cells in wort. A view of these is given in Fig. 139.

These chains of elongated cells partake of the character of an articulated mycelium. The capacity for producing such was first positively demonstrated by Hansen, and proves that the *Saccharomycetes* belong to the *Mycomycetes*, or *Eumycetes* with septate mycelia. Their position within this sub-kingdom was then, as already stated, indicated by their capacity for producing ascospores, which will be discussed in the next paragraph. In coloured nutrient media, such as beer wort and wine, the progress of film development is accompanied by a bleaching action, *i.e.*, the disappearance of colouring-matters. In this manner the colour of a wort can be slowly changed from dark brown to straw yellow.

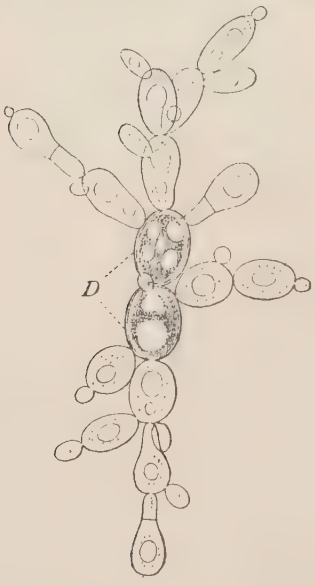


FIG. 139.—Pair of Permanent Cells

from the yeast ring of a six-months-old wort culture of Munich bottom yeast No. 2, and germinated to a well-developed chain of buds in a drop of wort on a microscope slide in sixty-four hours at 10° C. A septum has formed inside three of the members of the chain. Nearly all the cells exhibit one or two vacuoles, and the two permanent cells (D) show an even larger number. Magn. 750. (*After Will.*)

The film cells also differ strongly in their chemico-physiological behaviour from those of the sedimental yeast. The latter still develop in presence of an extremely low oxygen tension, and devote their chief energy to the decomposition of sugars. On the other hand, the metabolism of the film cells is indissolubly connected with the presence of a copious supply of oxygen. According to the results of investigations conducted on this point by B. RAYMANN and K. KRUIS (I.), they oxidise, to carbon dioxide and water, the alcohol continuously formed in the fermenting underlying solution, and degrade the albuminoids therein to amides and ammonium salts of organic acids. Formic acid and valerianic acid are also formed. Hence in this case the fermentative action of the sedimental yeast is replaced by respirative activity.

When submerged in fresh nutrient solution, these film cells produce vegetations, which finally behave just like normal sedimental yeast. The rate of morphological change and adaptation of physiological character differ with the species of yeast. In some, as was ascertained by H. WILL (VIII.), the characteristics appertaining to the film cells remain unimpaired in the first new generations, and in specially conspicuous cases several recultivations (repeated transferences of the crop to fresh nutrient solution) are required in order to produce a sedimental yeast equal in all respects to the original ancestors of the film cells used. Notice should also be taken, *e.g.*, of an observation on this point by ED. KAYSER (VI.). Further consideration will be given in subsequent paragraphs to this behaviour, from the standpoint of the theory of variation.

At present we have to deal with the consequences connected with the practical cultivation of yeast, namely, the restriction of film vegetation and the exclusion of cells derived therefrom. To effect this object it is necessary to keep the stock yeast in the laboratory under such conditions as are unfavourable to the development of film, without being at the same time inimical to the sedimental yeast. The appearance of the former may be counteracted by frequent transferences of the cultures to fresh nutrient solutions, and by keeping the culture at low temperatures. According to Hansen, the best storage medium for prolonged use is a 10 per cent. solution of saccharose. In this event, however, the sowing

should not amount to more than a trace. When, from any cause, the only yeast culture available for fulfilling an order is one that is already covered with a film, the same is suitable for direct transference to the large reproduction vessel, but must be first freshened up by preparing a reinoculation, which in turn is used to inoculate a fresh nutrient solution as soon as development is in full swing. The operation is several times repeated, according to circumstances, until one is able to assume that the film cells present in the first inoculation of sedimental yeast have been entirely suppressed. The beginner cannot be too strongly advised not to regard the task of yeast cultivation as completed by the preparation of the pure cultures, but rather to keep the latter under constant supervision, examination, and care. Neglect of these precautions, and, in the case under consideration, the use of sedimental yeast containing film cells, may, under certain circumstances, lead to irregularities in the progress of fermentation on the large scale, diminution in the quality (flavour, &c.) of the product, and hence to unpleasant consequences for the yeast cultivator. Cases in point have been recorded by A. Joergensen (VII.).

This, however, must not be held to imply that the film cells are the cause of all the unwelcome alterations that may appear in beer yeasts. On the contrary, other forces are here in operation; and from this side also, as already mentioned, we arrive at the wide field of variation in the yeast cell, a domain in which, as will be shown in a later chapter, Hansen, by his extensive experimental researches, has been our pioneer. Moreover, it should be recalled that Raymann and Kruis (I.) were able, by means of yeast derived from old film cells, to produce good beer that could not be distinguished from that obtained by the aid of normal yeast. This harmonises with the results obtained by Alb. Klöcker (privately communicated to the author) with Carlsberg bottom yeast No. I. and No. II., *Sacch. cerevisiæ I.*, Hansen, Marienthal yeast, and Will's No. 2 stock yeast.

Will's observations bring to mind the flying yeast (Flughefe) so dreaded by the brewer, *i.e.*, yeast cells which are of smaller size than those of the sedimental yeast, and, instead of settling, continue to swim in the beer, and thus retard clarification. This presumptive relation has not yet been more closely investigated, but the researches of Hansen and others have placed beyond doubt that this phenomenon is in many cases attributable to the presence of wild yeasts. Another point that requires closer examination is the part played, in the maturing of beer, by the film cells produced within the liquid. Finally, investigation from this point of view is also desirable on the problem of the cause of flocculence in the process of making pressed yeast by the new, so-called aeration or wort process, which differs chiefly from the Viennese method (§ 255) in the thick mash being replaced by a clear mash as nutrient medium; this, after pitching, being well roused by aeration, whereby the reproduction of the cells is strongly stimulated, accelerated, and increased. When fermentation is at an end the contents of the fermenting vessel are cooled and drawn off into large, flat clarifying pans, where the yeast crop settles down, and, after the removal of the supernatant liquid for distillation, is washed with water and finally forced into filter-presses, where it is brought into saleable condition. Occasionally the deposition of the yeast crop in the clarifying pans, and therefore its separation from the liquid, is obstructed by a so-called flocculence, which is characterised by the continued reascension of flocculent aggregations of cells from the deposit. The phenomenon has been described by Stenglein and Jörres in "Alcohol" (1892, p. 218), and also by O. Durst (I.).

§ 247.—The Ascospores.

The first observation of the ascospores in yeast cells was made by Th. Schwann (II.). He pointed out that these fungi reproduce in two ways: one being by the process known as budding, and the other by the formation, within the parent-cell, of daughter-cells, which are liberated when the membrane of the parent-cell opens. After this phenomenon had been described more closely by J. de Seynes (I.) in 1868, it was also observed a year later by M. Reess (III.) in cultures of beer yeast on boiled sections of carrots, &c. He found the process of development coincide with that of certain low *Ascomycetes*, and therefore classed these forms as ascospores, calling the mother-cells asci, and for this reason relegating the *Saccharomycetes* (in 1870) to the position of the lowest family among the *Ascomycetes*.

The earliest accurate investigations into the conditions under which this sporulation occurs were carried on by E. Chr. Hansen (XII.), and, apart from the general biological results, led to the important fact that we have here a reliable means, hitherto lacking, of separating the genus *Saccharomyces* into its species.

The conditions influencing the production of ascospores in the *Saccharomycetes* are given below: (1) To obtain energetic sporulation, it is necessary that the sample should consist of young and well-nourished cells. (2) The supply of air must be abundant. (3) The medium must be moist. (4) The temperature of the environment must be maintained within certain limits. (5) Within these limits the time required for the occurrence of sporulation is a function of the temperature. (6) Between the two extreme limits at which sporulation is still possible is an optimum temperature corresponding to a time minimum. The maximum temperature for sporulation is somewhat lower, and the minimum temperature rather higher, than for the phenomenon of budding.

To examine the individual conditions more closely. That the time within which sporulation occurs should be a function of the temperature requires no further analysis; but careful attention should be bestowed on the point (in 1) as to the condition of the cells, this being the prime factor determining the time limit. The time required for ascospores to be developed by any given species of *Saccharomyces*, kept at any given temperature, differs according to the physiological condition of the cells themselves. Hence, if it be desired to produce sporulation (unconditionally) in any given species, all that is necessary is to take cells that are in vigorous condition—a state attainable by repeated preliminary transferences into fresh nutrient solution. The case is, however, different when it is a question of determining the time required for sporulation to make its appearance at one or another temperature. In such event, it must be borne in mind that this time limit is a function, not merely of the temperature, but also of the physiological condition of the species under examination; consequently this latter factor must be eliminated in order to enable the influence of the former to be determined. Experience has shown that sporulation occurs earliest and most certainly when the cells have reached the culminating point of their reproductive (budding) and fermentative activity; and it is therefore in this condition that they should be employed for the experiment in question. On this account the cells to be examined for the time limit of sporulation should be subjected to the following preliminary treatment: the sample is sowed in sterilised beer wort and left to stand for several days at room temperature, Pasteur flasks being the best vessels for the purpose. A portion of the resulting sedimental yeast is transferred to fresh sterile beer wort and kept therein for twenty-four hours at 25° C., the fresh deposit being afterwards freed as carefully

as possible from the supernatant liquid, and employed for starting the spore cultures.

One example will suffice to show the necessity of taking the condition of the cells into consideration. It is afforded by Hansen's experience, and relates to *Sacch. Pastorianus I.* The culture was first conducted for a few days at room temperature, after which the sedimental yeast was retransferred, in the above manner, to two flasks, one of which was kept for twenty-four hours, the other for forty-eight hours, at 26° to 27° C. before starting the spore cultures. The following figures show the time required for the commencement of sporulation in the two cases :

Sacch. Pastorianus I., Hansen.

Sporulation occurred at	After previous Cultivation at 26° to 27° C. for :	
	24 Hours	48 Hours
29° C.	At the end of 27 hours	No Sporulation
28° to 27.5° C.	„ „ 24 „	At the end of 36 hours
23.5° to 23° C.	„ „ 26 „	„ „ 30 „
15° C.	„ „ 50 „	„ „ 54 „

The sample previously treated for forty-eight hours gave no sporulation at a temperature (29° C.) at which the other produced spores in abundance. Apparently this unfavourable effect is attributable to the increased alcohol content produced by the longer period of fermentation. The relation in question has also been observed by other workers, *e.g.*, by H. Müller-Thurgau (III.), A. Aderhold (I.), &c. The (artificially effected) temporary or permanent loss of sporogenic capacity of yeasts will be dealt with in the chapter on variation.

The best means of obtaining the moist medium and copious access of air essential to sporulation is afforded by the **gypsum block** proposed by Engel (I.) and suitably improved by Hansen. This block is a truncated cone, about 3 to 4 cm. in height, which is prepared, by means of an ungreased mould of sheet-iron, from a mixture of 8 parts, by volume, of powdered, calcined gypsum and 3 parts of water. After the block has become perfectly dry through long exposure to the air, it is placed in a covered glass basin enveloped in a double layer of filter-paper, and sterilised in the drying oven for an hour at 115° C. When cold, the yeast under examination is sown over the upper surface of the block, by means of a platinum loop, previously sterilised by heating to redness. The glass basin is then charged with sufficient sterilised water to quickly moisten the block completely, from below upwards, and in addition leave a layer of water about 1 cm. deep in the bottom. The cover glass should not fit quite tight, but is preferably supported on an uneven bearing, so as not to exclude the admission of air. The arrangement of the whole is shown in Fig. 140, about one-half or one-third the real size. The block, in its basin of water, is then placed in a thermostat, maintained at the desired temperature for the experiment.

The nature of the material composing the moist medium also influences the time limit of sporulation. Since the necessary cleaning of the gypsum block, by brushing with water after each experiment, gradually destroys the block itself, H. Wichmann (III.) proposed to replace gypsum by solid cubes of firebrick. It was, however, shown by J. Chr. Nielsen (I.) and Alb. Klöcker, that sporulation took longer on this substance than on gypsum ; on the other hand, equal results with the latter are furnished by the clay cubes first employed by H. Elion (III.).

Th. Bowhill (I.) uses gypsum blocks of the same shape (a cylinder, cut through slantwise) as the slices of potato employed for streak cultures in test-glasses; and he likewise uses these glasses. No advantage accrues from the substitution of filter-paper for gypsum blocks recommended by E. Wasserzug (II.).

The conditions with regard to access of moisture and air are also fulfilled by streak cultures on solid nutrient substrata; and, as a matter of fact, ascospores are formed under such circumstances—as was first observed by Hansen, and afterwards by other workers. For example, the part of the gypsum block can also be played by a damp wall (in the brewery) towards the yeast sprinkled thereon, or by a damp filter-bag, &c., for yeast gaining access thereto from the air. Again, as is shown in Fig. 141, spores may occasionally be observed in the development of film; but these are merely exceptional instances. Their occurrence was vainly sought by H. Will in bottom-fermentation beer yeasts. On the other hand, these organs are, naturally, not lacking in the films of the comparatively few *Saccharomycetes* that grow almost exclusively in the form of film and not as sedimental yeast, a special instance being afforded by *Sacch. membranæfaciens*. Sporulation has also often been observed in the interior of liquid cultures: by Hansen in repeatedly aerated cultures of several species in yeast water, and in *Sacch. Ludwigii* grown in 10 per cent. saccharose solution; also by H. Will (VIII.) in a wort culture of his bottom (beer) yeast No. 93; by P. Röser (II.) in a 1 *per mil* solution of peptone; and by Hautefeuille and Perrey (I.) in a culture of wine yeast in must at 28° C. When the nature of the question at issue necessitates absolute purity of the spore culture, Hansen makes the sowing on a thin layer of water at the bottom of a flask, and not on a gypsum block in a glass basin.

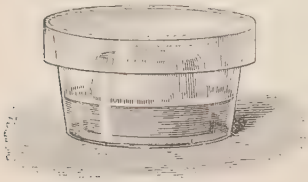


Fig. 140.—Gypsum-block Culture.

With regard to the inception of sporulation we know comparatively less than we do of the conditions under which the phenomenon occurs. In general it may be said, according to the determinations made by E. Chr. Hansen (XXVIII.), that the cells do not resort to sporulation until they are unable to reproduce further by budding. Both old and young cells are capable of producing spores, even such as have not put forth any buds at all. In the case of Wortmann's wine yeast Johannisberg II., for example, this result can be obtained by placing the young cells in a saturated aqueous solution of calcium sulphate, whereby budding is suppressed and sporulation begins. Compare what has been stated in the last paragraph but one of § 223.

The progress of development in the ascospores will now be followed in the case of *Sacch. cerevisiæ I.*, Hansen. If, at the end of about twenty-four hours after the streak has been laid on the block and the latter placed in the thermostat at 25° C., a small portion of the yeast streak be scratched off with a clean glass rod or a needle, distributed in a single drop of water, and examined with a strong power (300 to 500) under a cover-glass, decided indications of incipient sporulation will be observable in a larger or smaller number of the cells. The plasma will be found to have separated itself into a number of balls, as shown at *a–d* of Fig. 142. Each of these globules soon becomes covered with a membrane, so that ripe spores (*f–j*) will be visible after another twenty-four hours. With regard to the behaviour of the cell nucleus during this period, fuller particulars will be given in § 250. It may be remarked that a portion of the plasma in the parent-cell (ascus) remains over, and holds the spores somewhat together.

A careful sorting of the samples will quickly show that considerable differences exist with respect to the dimensions of the spores, and that too not merely

between those in different cells, but also in those present in one and the same ascus. The number (2 to 9) of the spores in the different asci will also be found to vary. What has already been said of the cells of sedimental yeast also applies to the ascospores: neither form nor dimensions is a reliable indication of species, and therefore these characteristics cannot serve unaided for their specific differentiation. It is only in special instances that this is possible, namely, when a species of the group *Sacch. anomalus* is present. The first representative of this group was discovered by Hansen (XVII.) in a Bavarian stock yeast, and it was he who gave it the foregoing specific appellation. This new species differs from those we have known hitherto, inasmuch as the ascospores, instead of being perfectly globular, are minus a more or less considerable portion of the sphere; and as the margin of this flat or slightly arched terminal surface is surrounded by a projecting border, the whole ascospore has the form of a hat (*see* Fig. 143). In the course of time other species producing similar ascospores, but differing in other respects, have been discovered, so that at present we have to deal with a

Fig. 141.—Wine Yeast from Walporzheim.

Assemblage of buds from an old film; some of the members have produced spores. Magn. 800. (*After Aderhold.*)

Fig. 142.—Sacch. cerevisiæ I., Hansen.

Ascosporulation. Magn. 1000. (*After Hansen.*)

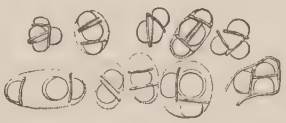


Fig. 143.—Saccharomyces anomalus, Hansen.

Ascospores. In three instances these have already been set free. In one the membrane of the parent cell is just breaking; in the remainder this has not yet happened. Magn. 1000. (*After Hansen.*)

whole group of such Saccharomycetes, which will be reported on more fully in a later section. More will also be said later on about the special method of sporulation observed by H. Schiönning (I.) in *Schizosaccharomyces octosporus* and possibly to be regarded as a sexual act.

When it becomes necessary to express in figures the time limit for sporulation, the moment selected is that at which the first appearance of spores becomes visible. To wait until the period of ripening is, on the other hand, impracticable, there being no reliable means of determining when that state is attained. Taking the first-named as the critical moment, Hansen (XII.) was the first to determine the relation between temperature and the time limit of sporulation of six species of *Saccharomycetes*. Afterwards a series of workers made similar determinations with a large number of species: H. Will (VIII., IX., XVII.), J. Chr. Holm and S. V. Poulsen (II.), with European beer yeasts; A. Laschié (IV.),

with American beer yeast; L. Marx (I.), Ed. Kayser (VII.), and the latter along with G. Barba (I.), on French wine yeast; R. Aderhold (I.), on German wine yeasts; A. Nastukoff (I.), on Russian wine yeasts; and W. Seifert (I.), on Austrian wine yeasts; also by E. Kayser (III.), on French fruit-wine yeasts; and by Chr. Grönlund (I.), J. Chr. Nielsen (I.), and Alb. Klöcker (I.), on several species still to be mentioned. The main object of such investigations is the determination of the minimum, maximum, and optimum temperature of sporulation; and several complete series of similar data have been compiled. The relation between the height of the thermometer and the time limit of incipient sporulation in a given species may also be graphically expressed by plotting the temperatures as abscissæ and the corresponding time-limit values as ordinates of a rectangular system of co-ordinates, and connecting the points of intersection by a line. Such lines are mostly termed sporulation curves in the literature of the subject.

The percentage ratio of the number of sporogenic cells to the total cells present differs greatly according to the species, origin, kind of previous nutrition, &c., but in most species fails to attain even approximately to 100. In many of the cultivated yeasts, *e.g.*, five out of thirty-two species of bottom-fermentation beer yeasts examined by Laschié (IV.), no success has, so far, attended the attempts made to bring about sporulation; consequently for the present these cannot be regarded as *Saccharomycetes*.

On the basis of his experimental results in connection with sporulation, already alluded to, Hansen has worked out a method of biological analysis for brewery stock yeasts; and this method has been further developed, especially by J. Chr. Holm and S. V. Poulsen (I. and II.).

The term "culture yeast" is applied to such yeasts as have been cultivated for practical use in fermentation technology, and have then been employed therein. More than this is not implied by the expression, nor does it apply in any way to their origin. On the other hand, the yeasts met with wild in nature are termed generally wild yeasts. Many of these are harmless, though others, as already mentioned in § 245, are capable of producing disturbances on gaining access to the operations of brewing, &c., and are then spoken of as (technically) pathogenic. Examples of this class are afforded by *Sacch. Past. I.*, Hansen, which imparts a repulsive bitter taste to beer; also *Sacch. Past. III.*, H. and *Sacch. ellips. II.*, H., which produce haze. As a further deduction from the observations of Hansen that the wild yeasts in general form ascospores more quickly and abundantly than the bottom-fermentation (culture) beer yeasts, Holm and Poulsen have shown that the latter can be classified into three groups according to their behaviour in this respect. The members of the first group remain sporeless, both after seventy-two hours at 15° C. and after forty hours at 25° C. Those of the second group, whilst remaining sporeless after forty hours at 25° C., exhibit spores after seventy-two hours at 15° C. Finally, the members of the third group behave in an exactly converse manner to those last mentioned. On the other hand, the wild yeasts exhibit spores under both sets of conditions (15° C. and 25° C.) within the time limits specified (seventy-two and forty hours respectively), or even much earlier. Consequently the desired differentiation can be obtained in all cases by making parallel tests at 15° C. and 25° C.

According to Hansen's observations—which we shall have to deal with in the paragraphs treating of mixed sowings—the wild yeasts that have crept into the pitching yeast or the wort first make their appearance in large quantities towards the end of primary fermentation in the upper portions of the contents of the fermenting vessel. Consequently at this period a sample should be drawn from this part in a glass; the suspended yeast cells must be left to subside and then immediately transferred to the prepared gypsum blocks, which are maintained at

the temperatures of 15° C. and 25° C. respectively. At the end of forty and seventy-two hours respectively a sample is taken of each, and should spores be detected in either or both series, then the presence of wild yeasts is demonstrated. When the brewery is unprovided with a suitable laboratory and an expert, a sample of the sedimental yeast must be sent to a laboratory, a drop of the yeast being dried on blotting-paper in the manner described in a later paragraph dealing with this matter. Owing to the abundant sporulation of the wild yeasts, this method is very decisive, Holm and Poulsen having by this means succeeded in detecting the presence of as little as about ½ per cent. of added wild yeast (one or other of the above-named three pathogenic yeasts) in twenty different species of bottom-fermentation beer yeasts. For practical use this is sufficiently delicate. On the other hand, to be of any value for testing yeast in a pure-culture apparatus, a method must be absolutely reliable and capable of detecting and isolating even the slightest trace of infection. With this object the yeast from the apparatus is subjected to a preliminary treatment, by means of which the amount of any wild yeast present in the sample is increased. This matter will be further described in a subsequent paragraph treating of the influence of organic acids on yeasts.

Differences also exist between the cultivated bottom-fermentation beer yeasts and the wild yeasts, in connection with the structure of the ascospores. Those of the latter are relatively smaller, and their contents are homogeneous and of high lustre, whereas the ascospores of the culture yeasts mostly exhibit vacuoles and granulation, and the membrane is clearly discernible.

The examination of the top-fermentation beer yeasts, distillery yeasts, and pressed yeasts by this process is a more delicate operation, because they sporulate much earlier and more abundantly than the bottom-fermentation yeasts; for instance, a species from the Munich Brewing Station, examined by Will, produced spores in fourteen hours. Nevertheless, according to JOERGENSEN (VIII.), the test is applicable if low temperatures (12° C.) be employed. The wine yeasts sporulate still more rapidly, 46 out of 58 examined by Marx being found to produce spores within twenty-four hours at 25° C. Similar results were obtained by Aderhold and by Nastukoff. The proposed supercession of spore analysis by the small-drop culture method introduced by P. LINDNER (XV.), will be referred to later, in a paragraph dealing with pure culture applied to yeasts.

When the gypsum block cultures are left exposed for a long time they become infested with all kinds of amœbæ, which gain access from the atmosphere and grow by devouring the yeast, as was observed by P. LINDNER (VI. and X.). In such event, sporocysts, the permanent form of these animalculæ, can be discovered on the blocks.

§ 248.—Germination of Saccharomycetes Spores. Their Resistance to Heat. Pasteurisation.

REESS (I.) was the first to observe the germination of *Saccharomycetes* ascospores, and more extensive and precise investigations were afterwards conducted by E. CHR. HANSEN (XVII.), whose reports constitute the main basis of the information now given.

Speaking generally, the germination of spores proceeds in one of four different ways. An example of the first and most usual method is afforded by *Sacch. cerevisiæ I.*, Hansen. The ascospores of this yeast are capable of germinating as soon as ripe, unless prevented by unfavourable external circumstances. The presence of water is an **indispensable condition**, large quantities of this liquid, and of any nutrient substances present, being taken up by the germinating spores, which thereupon swell up to a considerable size. If they are still enveloped

by the parent-cell, this latter is stretched and assumes a somewhat angular contour, as shown at Fig. 144 at *a* and *d*. The residual plasma in the parent-cell is compressed flat between the spores, but reacts on the latter, and forces them into a polyhedral shape so long as the membrane of the parent-cell continues to hold firm. Consequently the ascus looks as though divided into compartments. In some instances (see *g*) a coalescence of the spore membranes occurs at the points of contact. In the case illustrated, the parent-cell contained three spores fused into one by the coalescence of their membranes. On pressing the cover-glass this fused aggregation was expelled from the parent-cell, the membranes of the three compound spores bursting simultaneously, each in one place. A similar case is illustrated at *e*, but with this difference, that here the parent-cell contained four spores, one of which remained behind after the application of pressure, the parent-cell having contracted to its original dimensions. Even without artificial aid the membrane of the parent-cell finally gives way under the growing pressure and liberates the spores, which then put forth buds (*f*) and henceforward behave like vegetable cells. The extent of subsequent budding naturally depends on the external conditions (nutrient media, admission of air, temperature, &c.). Two examples are shown in Fig. 145. In some instances the septum formed by the coalescing membranes of two spores is absorbed and a fusion of the contents of both spores occurs. The ascospore germination of all the yeasts employed in brewing, distilling, and wine-making follows this first typical course.

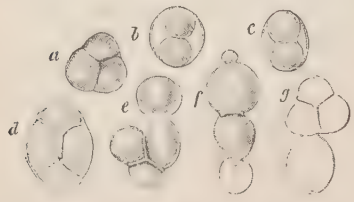


FIG. 144.—Saccharomyces cerevisiæ I., Hansen.

Germination of spores. For explanation see text. Magn. 1000. (*After Hansen.*)

An example of the second method of spore germination is afforded by *Sacch. Ludwigii*. Various genera of deciduous trees, the oak in particular, frequently exhibit a mucinous flux which is readily perceptible with a little care. In the

FIG. 145.—Saccharomyces cerevisiæ I., Hansen.

a. Three coherent spores issued from the parent-cell. *a'*. The same after nineteen hours in wort at 20° C. in the Ranvier chamber. Two of the spores carry each a single bud. *a''*. After twenty-two hours; *a'''*. after thirty hours.

b. A parent cell with four spores; the two below are partly covered by the upper pair. *b'*. After eighteen hours (as with *a'*). The membrane of the parent-cell has become torn, and hangs, like a veil, on the colony of cells formed from the spores. Magn. 1000. (*After Hansen.*)

earliest stage of the complaint there exudes from the bark—both from frost cracks, knot-holes, and apparently uninjured places—a white froth, the smell of which is enough to show that alcoholic ferments have contributed to its formation. For more detailed investigations on the phenomenon we are indebted to FR. LUDWIG (II.). This exudation, which may also prove of interest to foodstuff chemists in connection with birch wine, was recognised by him as the field of activity of various species of micro-organisms, the occurrence and action of which he traced in several directions. One of the fungi present and identified as exciting alcoholic fermentation in this exudation was then examined by E. CHR. HANSEN (XXIX.) and named *Sacch. Ludwigii* in honour of its discoverer. This fungus is interesting in many respects, *inter alia* as a very fine example of mycelial film and septation in the cells of same. At present, however we have

to do with another peculiarity, namely, the behaviour of its endospores during germination. These spores, instead of putting forth rounded buds of a shape similar to their own, as in the type just described, produced an elongated, sausage-shaped promycelium of the kind illustrated in Fig. 146, g''', h'''. This promycelium it is that puts forth the buds, and it therefore constitutes an intermediate stage between the bud cells and the spore. Contrary to the usual procedure, the yeast cells of normal shape produced by this mycelium are not liberated as the result of constriction, but by the development of a septum at the place where separation afterwards occurs. This is shown at g''''. Very often, however, the promycelium is not the result of the germination of one single spore, but of the

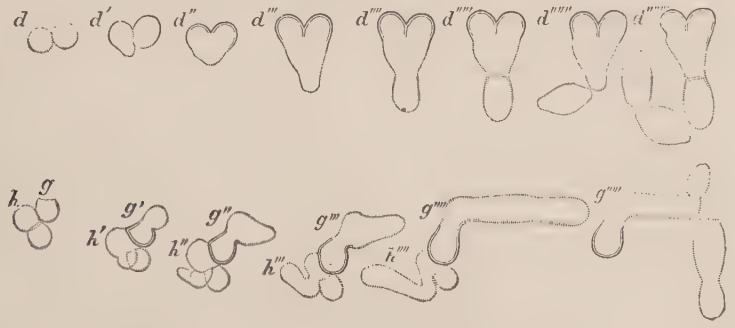


FIG. 146.—Saccharomyces Ludwigii, Hansen.

d. Two spores, germinating on the one side (d'), fusing (d''), growing into a promycelium (d'''), putting forth a bud (d'''') at the crown, and separating by the development of a septum (d'''''), whereupon one of them (d'''''') assumes a rounded form, which procedure is repeated several times (d''''''').

g, *h*. Two spores, each growing to a tube which produces daughter-cells independently, without coalescence. Magn. 1000. (*After Hansen.*)

coalescence of two germ tubes to form one new cell. This process, one of actual cell fusion, is shown at d–d''''''', where the two spores can be seen germinating on the one side, each in an elongated form (d'), after which the two fuse together (d'') to a single cell, which in turn grows out at one side to form a promycelium (d''''). A daughter-cell (d''''') then separates and quickly assumes the lemon shape (d'''''') characteristic of this fungus. The cell fusion occurring in this instance differs from the previously described type in two ways: there it was the exception, here it is the rule; there it took place between the spores themselves, here only in the germ tubes proceeding from the spores. This fusion is not of invariable occurrence, but is occasionally hindered by the tubes (g''') taking different directions from the start, or by the premature withering of one or the other of them; or sometimes only one is formed, the adjacent cells being prevented from germinating by circumstances, *e.g.*, age. Apart from *Sacch. Ludwigii*, this method of spore germination is only known to occur in one other species of *Saccharomyces*, which was discovered by J. BEHRENS (VIII.) on fresh hop cones.

The third method of germination is found in the group *Sacch. anomalus*, and differs from the first type solely in the influence exercised by the peculiar shape of the spores. Three examples are illustrated in Fig. 147. These relate to sowings of spores taken from old gypsum-block cultures and grown in beer wort in the Ranvier chamber, the top row being kept at 28° C. and the other two at 23° C. The germinating spore swells up, and gradually puts forth a number of buds at various points of its surface, these in turn reproducing by budding. During this operation the hat-brim-like projection round the spores disappeared in the middle series (*b*), but remained in the other two (*a* and *c*).

A fourth type of spore germination is exhibited by *Sacch. guttulatus*. This fungus is an invariable inhabitant of the contents of the stomach and intestines. It also occurs in the excrement of the full-grown rabbit, and was first discovered therein by R. Remack in 1845. It was classed along with the yeasts by Robin in 1847, under the name *Cryptococcus guttulatus*, and finally recognised as a *Saccharomyces* by Buscalioni, in 1896, as the result of a searching investigation. In this species, which does not bud until about 37° C., but is capable of sporulation at about 14° C., and thus forms in this respect an exception to the rule given in the preceding paragraph, the sporulation proceeds in the manner

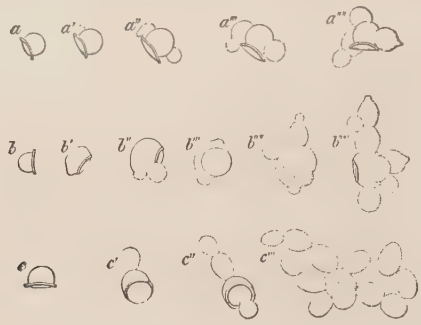


Fig. 147.—Sacch. anomalus, Hansen. Spore germination.

a. A spore at the commencement of germination, with the brim turned towards the left; *a′* is the same after seven hours, one bud having been formed; *a″* is the same after twelve hours, a second bud appearing on the spore; *a‴* is after fifteen hours; and *a⁗* after twenty hours.

b. A spore with the brim turned towards the right; *b′* the same after ten hours; *b″* after twenty-one hours; *b‴* at the end of twenty-four hours; *b⁗* after twenty-five hours; *b⁗′* after twenty-seven hours, the spore with its daughter-cells having turned through an angle of 180° in the nutrient solution.

c. Is a spore with the brim turned downwards; *c′* the same after eight hours; *c″* after ten hours; *c‴* after twenty-four hours. Magn. 1000. (*After Hansen.*)

described in §§ 57 and 58. As was first demonstrated by Arm. Wilhelmi (I.), the contents of the elongated germinating ascospore envelop themselves in a new membrane, the resulting germ then splitting the old membrane, either at the end or laterally. In the act of swelling it also bursts the membrane (if still intact) of the parent-cell, escapes, and immediately begins to bud. An illustration of this process is given in Fig. 148.

The spore, however, does not invariably germinate, but, as was ascertained by Hansen (XXVIII.), may, under certain circumstances, itself play the part of an ascus and develop ascospores (of the second order) in its interior, instead of reproducing by germination and budding. This end may be attained by allowing the ripe spore to swell up in a solution of sugar, and then transferring it to a saturated aqueous solution of gypsum.

The ascospores of *Saccharomycetes* exhibit greater powers of resistance to external influences than is possessed by the vegetative cells. Reference will be made in subsequent paragraphs to cases wherein the extraction of moisture, drying, or chemical stimulants come under consideration as influences of this nature. At present we are mainly concerned with the effects of temperature, and that too in presence of liquids. Hansen (XII.) was the first to point out that the spores of *Sacch. ellipsoideus II.* perish in five minutes when kept in sterilised water at 66° C., though they survive this period at 62° C. On the other hand, the vegetative cells taken from a two-days-old culture in beer wort at 27° C. perished within five minutes on immersion in water at 56° C, although

they could stand 54° C. In the case of *Sacch. Past. I.*, the corresponding values obtained were 62° and 58° C. for the spores, and 54° and 52° C. for the young vegetative cells. The resisting power of old vegetative cells, the permanent cells especially, approximates more closely to that of the ascospores. Greater tenacity of life was exhibited by a species (*Sacch. Zopfii*) isolated by Zopf from the sugar juice in a Silesian beet-sugar works, which species was more closely examined by A. Artari (I.), and does not perish below about 67° C., under identical conditions. And still greater resistance is offered by the *Saccharomyces* mentioned in the preceding paragraph as Will's (IX.) No. 811 yeast, which was discovered in a beer of repulsive, bitter, irritant flavour, of which malady it was recognised as the cause. When kept in beer wort at 70° C., an exposure of half an hour was required to kill the vegetative cells, whilst the spores withstood the influence of 75° C. for the same period. Antithetical to this is *Sacch. Joergensenii*, which was discovered by A. Laschè (I.) in a very turbid American, so-called temperance ale, and quickly perishes at temperatures above 30° C. Similar experiments were made with French wine yeasts by E. Wasserzug (II.) and southern wine yeasts by Ed. Kayser (VII.), the latter of whom found the fatal temperature for spores to be about 5° C. higher than for vegetative cells.

Fig. 148.—Saccharomyces guttulatus.

Two ascospores, the contents of which have enveloped themselves with a new membrane whilst still enclosed in the intact membrane of the parent-cell (ascus). The old spore membrane was ruptured terminally in the one spore and laterally in the other. Both germs still partly invested by the old spore membrane. Magn. 1066. (*After Wilhelmi.*)

In order to ensure stability in fermented beverages it is sufficient to so far weaken the yeast cells by warmth as to preclude subsequent reproduction, and thus practically eliminate them. To attain this end, experience has shown that the necessary influence must be less stringent than is required for killing the cells; and the case is facilitated when the liquid contains substances which, like alcohol and acids, exercise an injurious effect on yeasts and bacteria at a somewhat higher temperature than the normal. These latter conditions are fulfilled in the case of wine, beer, and wine-must, and an explanation is thus afforded of the fact that these liquids can be converted into a stable condition by a gentle heat, considerably below boiling-point. The earliest application of such a process is traceable to the Japanese. According to O. Korschelt (I.), the Japanese rice beer, or *saké* (already mentioned in an earlier paragraph), is made to keep through the hot summer months by warming, a practice that has been pursued for more than a century. The first to adopt such a method in Europe was Scheele in 1782 (§ 11); and Appert, in 1810, recommended the warming of wine, in corked bottles, to 75° C. It was, however, soon found that this treatment injured the fine flavour, especially in the case of red wines. Then, in 1865, Vergnette-Lamotte (I.) proposed to employ a temperature not exceeding 50° C., thereby avoiding the evil specified without missing the principal object in view; and at the same period Pasteur (XX.), as a result of his researches on wines, found that they could be reliably protected against deterioration by warming them up to 55° to 60° C. With regard to the priority dispute between the two authorities, the reader is referred to the bibliography. Thanks to the reputation at that time enjoyed by Pasteur, the process patented by him was quickly introduced into practice, and called Pasteurisation in his honour.

The inventor's primary intention was to use this process merely for imparting

stability to wine, the killing or permanent weakening of the germs therein being effected by the conjoint influence of a temperature of 55° to 60° C. (in itself insufficient for the purpose) and of the toxic action (at this temperature) of the alcohol and organic acids present. Subsequently the name was extended to the attempts made, by gentle warming, to weaken or kill the germs in other liquids, *e.g.*, milk (§ 128), wherein these chemical auxiliaries are lacking. This important difference has not been sufficiently appreciated in the literature, and is not infrequently ignored in practice, the consequence being failures and illusory results. So far as concerns the pasteurisation of wine, beer, and must, which is all that we have to consider in this place, the technology of the process (which is practised in a variety of modifications) must be dismissed in a few words, the reader being referred, for further information, to the descriptions given by Pasteur (XIV.), Babo and Mach (I.), and F. Malvezin (I.). The forms of apparatus latterly constructed for the pasteurisation of wine have been described by Vassillière, Charvet and U. Gayon (I.), the last-named of whom (I.) has also made comparative examinations of several systems. Illustrations and descriptions of the apparatus designed for pasteurising (or, as it is often erroneously styled, sterilising) bottled beer, will be found in Fassbender's "Technologie," and in the handbooks mentioned in § 81.

The difficulties encountered in the practical pasteurisation of wine, beer and must are mainly of three kinds. First is the selection of the lowest temperature at which it is possible to attain the end in view, viz., to kill the yeast and bacteria, or render them incapable of setting up any further action in the liquid in question. This temperature, however, necessarily varies according to the species of the organisms present, and the chemical composition of the wine or beer. Hence no invariably applicable figure can be given, though 50° to 60° C. may usually be adhered to. The further this limit is exceeded in the direction of the boiling-point, the more decided will be the boiled—or, in the case of beer, the so-called bread-like—flavour of the liquid treated. This flavour largely owes its origin to the modification of certain constituents of the hot liquid by oxygen. To afford a remedy—and this forms the second difficulty—not only must the access of air be prevented, *i.e.*, by warming the liquid in corked bottles or bunged casks, but also the oxygen present in solution has to be eliminated—as was proposed in H. Gronwald's German Patent, No. 98,584 (1896). The third difficulty, which we need not now consider, resides in the formation of a coagulum produced by the heat, and constituting a deposit which, since it would spoil the appearance of the wine when poured out, has eventually to be removed by filtration, followed by a second pasteurisation.

Some very tenacious bacteria are able to survive the pasteurisation of the three liquids mentioned, but are scarcely capable of doing any subsequent damage. Thus, in a sample of Munich beer sufficiently pasteurised for the export trade, Dömens (I.) found 34 bacteria per c.c., all able to develop on nutrient gelatin. It may be remarked that the *Mycoderma* are very susceptible to this treatment; and it has been proved that, under the conditions prevailing in the pasteurisation of beer, a temperature of 60° to 65° C. is fatal to six different species, including *Mycoderma rubrum* and *M. humuli*; as also to *Saccb. Past. III.* and several species of beer yeast (Lasché (V.)). In the case of several species of mucinous yeasts examined by Richard Meissner (II.), the limit was found to range between 51° and 61° C. according to the species.

Since it is easier to render the yeasts innocuous than the bacteria, a slighter warming will suffice when there is reason to believe that the latter are entirely absent from, or harmless in, the wine under treatment, and when, consequently, the task is confined to eliminating the yeasts alone, in order to prevent them afterwards setting up secondary fermentation and turbidity. Carl Schulze (I.)

has shown that, under these circumstances and in presence of about 10 per cent. of alcohol, the yeasts are killed by exposing the bottled wine to a temperature of 45° C. for two hours. The samples tested in this manner did not exhibit any alteration of flavour, though a precipitation of coagulum occurred.

The preparation of so-called unfermented and non-alcoholic grape and fruit wines, which in reality are nothing more than stabilified grape or fruit must—and which, thanks to the temperance movement, are more and more coming into favour—has also benefited by the observations just recorded. H. Müller-Thurgau (XII.) in particular has occupied himself with this question, and has drawn up practical instructions for pasteurising these beverages, by exposure to a temperature of 60° C. for half an hour to an hour. Here also it is necessary to filter off the resulting deposit, and pasteurise again. Fruit juices, *e.g.*, the cherry juice so highly appreciated in North America, can also be rendered stable by similar treatment.

The reader will now be in a position to understand and thoroughly appreciate the operation of warming the curd in cheese-making (as mentioned in § 182), and the influence of the temperature then maintained on the subsequent progress of ripening.

CHAPTER XLVII.

ANATOMY OF THE YEAST CELL.

§ 249.—The Cell Membrane.

In the case of younger cells, actively engaged in metabolism, the **cell membrane** is very thin (only a few tenths of a μ) and has nearly the same refractive power as the enclosed plasma. It may be rendered clearly visible and distinguishable by allowing some strongly plasmolytic reagent, *e.g.*, dilute acids, to act on the cell, whereby the contents are caused to shrink. The membrane can be exposed in a simpler manner by merely pressing gently on the cover-glass of the microscopical preparation, so that the cells burst and the empty membranes become visible as pale skin. The membrane of the permanent cells described in § 245 and § 246 is, however, discernible without this preliminary treatment. According to the researches of H. Will (VIII.), the membrane in these cells attains a thickness of 0.7 to 0.8 μ, or even 1 μ occasionally. This thickening of the cell membrane is a precautionary measure against adverse influences, and occurs more particularly when the yeast cells are obliged to develop in or upon a concentrated nutrient medium, *e.g.*, strong ale ("Bockbier") wort or wort-gelatin. C. Becker (I.) determined the thickness of the cell membrane of stock yeast for ordinary Munich beer as 0.5 μ, whereas that taken from a vat of strong ale measured up to 0.7 μ, and that from the still stronger Salvator beer 0.9 μ. The increase in thickness is accompanied by diminished penetrability, and consequently decreased fermentative power. For this reason the sedimental yeast from strong ales is rarely used again, or only for pitching worts of lower gravity (lager beer).

The influence of dilute acids (*e.g.*, a 1 per cent. solution of osmic acid) or alkalis, on yeast cells, especially the permanent cells, is evidenced by **stratification of the membrane**, which was first observed by H. Will (VIII.) and then confirmed by O. Casagrandi (I.). Mostly the membrane is divided into two layers, or in some instances into three or even more; though C. Becker (I.) never succeeded in finding more than two. The outer of these gradually becomes detached as the cell grows older, a phenomenon first observed by P. Lindner (VI.) in the case of a Torula. An instructive example of this shedding the membrane is afforded by Fig. 149. The separation (*a*, *b*) may either begin at one spot and then gradually extend to a progressively increased peeling of the outer layer (up to 0.5 μ thick), and the exposure of the inner and more delicate stratum of the membrane; or it may go on simultaneously all over the surface, so that the cell remains enclosed in the unbroken outer layer as in a bag (*c*), through which any daughter-cell that may be produced has to make its way. Apart from such cases the membrane of yeast cells is invariably closed on all sides. It is necessary to emphasise this in view of Bizzozero's (I.) improbable report that the membrane of a budding fungus found by him in human epidermis—and of still indefinite position in the botanical system—was full of pores.

More or less perfect fragments of such discarded layers of cell membrane are found in almost every liquid yeast culture of a certain age. There is no danger of their being mistaken, even by a beginner, for the albuminous flakes so common in beer-wort cultures, if microchemical reactions be called in aid, since these will

quickly show that we have here to do with a substance differing both from albumen and from cellulose.

With regard to the chemical composition of the materials constituting the membrane of the yeast cell, there is not much reliable information to offer at present. Leaving out of consideration what, in the present state of our knowledge, must be regarded as an erroneous counter-opinion expressed by J. Schlossberger (I.), the absence of true cellulose therein was ascertained by Mulder (IV.), and afterwards by C. von Wisselingh (I.). The absence of any appreciable action of

Fig. 149.—Permanent Cells of Bottom-fermentation Beer Yeast.

a. The outer layer of the membrane beginning to peel off. Cell contents rich in drops of oil. From a culture in beer wort.

b. The cell has perished from some unknown cause, before the bud in course of formation had fully developed and separated therefrom. The dead plasma has collected in lumps. The outer layer of the membrane has almost completely loosened, and encloses the cell like the calyx does a flower.

c. A budding cell from which the exterior layer of membrane has become detached on all sides. In this bag, wherein two daughter-cells are just commencing to separate, rests the parent-cell, which is beginning to put forth a third new daughter-cell through a crack in the outer membrane. *b* and *c* were taken from the yeast ring of an old culture in peptone-dextrin-nutrient-salt solution. Magn. 2000. (*After Will.*)

ammoniacal copper oxide on the yeast cell had already been observed by Liebig. According to the concordant results furnished by the researches undertaken by C. von Wisselingh and by Tanret (III.), chitin, the second material taking part in the structure of the cell membrane of the *Eumycetes* in general, also seems to be lacking in the case of yeasts. The alleged observation, by Curtis (I.), of a violet coloration produced by zinc iodochloride on the membrane of a pathogenic budding fungus, was declared by Casagrandi to be erroneous; and no more than a faint yellow coloration is produced in the membrane of vegetative yeast cells by simple iodine solution. According to H. Will (IX.), the report made by several observers to the effect that the spore membrane of various yeasts is strongly tinged a yellowish brown by zinc iodochloride, must be corrected in the sense that the seat of this coloration is not in the spore membrane itself, but in an overlying unconsumed residue of the plasma of the parent-cell. This Will was able to determine more particularly in the case of two species of wild yeast examined. According to Lindner (VIII.), the spores of *Schizosaccharomyces octosporus* exhibit a very peculiar behaviour in this respect, a powerful blue coloration being imparted by iodine and potassium iodide to the spore membrane, and often to that of the vegetative cells as well. The same behaviour was observed by J. Ch. Holm in another species allied to the one just mentioned. Along with these exceptions must also be classed the blue coloration observed by Liebermann and Bitto (I.) on the addition of zinc iodochloride to a macroscopical preparation of so-called yeast cellulose (§ 225). In connection with this behaviour of the yeast-cell membrane towards colouring-matters, Casagrandi remarks that Congo Red is not absorbed, whereas, on the other hand, very good staining can be effected with Ehrlich's Methylene Blue or Hanstein's Aniline Violet. C. Becker (I.),

however, found only the latter of these two effective. Solution of the membrane occurs on the cells being immersed in concentrated chromic or sulphuric acid, whilst other acids are ineffectual. The same applies to the mixture of nitric acid and potassium chlorate, known as Schulze's maceration liquid, through its mode of action having first been observed and utilised by Brunnengräber in the laboratory of Professor F. F. Schulze at Rostock. This is related by A. Tschirch (IV.) in an admirable historical review of microchemical methods.

The foregoing, together with a series of other microchemical reactions, led Casagrandi to the belief that the yeast-cell membrane contains a substance chemically allied to the pectin recognised by Mangin as a constituent of other vegetable membranes. It is, however, difficult to ascertain whether and how far this view is correct, owing to the absence of means for detecting this carbohydrate. Still more undecided is the question as to the divergent chemical composition of the various strata of the membrane. That some difference does exist may be deduced from the influence exerted by chromic acid (diluted with its own volume of water) on the cell membrane, whereby the inner layer is more readily dissolved than the outer, especially in the case of permanent cells. This observation was first made by H. Will, and was afterwards confirmed by Casagrandi and supplemented by different staining tests. It was probably owing to a similar observation that Nägeli was led to surmise that the cell membrane in the films of Mycoderma is cuticularised.

The hypothesis founded on the microchemical examination of the yeast-cell membrane is undeniably supported by the macrochemical researches of Salkowski (VI.) on the same point. He found that the residue obtained by extracting pressed yeast for half an hour (§ 254) with boiling 3 per cent. caustic potash solution, and amounting to about 3.1 per cent. by weight of the substance taken, was separable into two components—one soluble, the other insoluble—by prolonged boiling in a large volume of water, under a reflux condenser, or in the autoclave at a pressure of 2 to $2\frac{1}{2}$ atmospheres. On separating and concentrating the solution, the soluble constituent was precipitated by absolute alcohol, as a white powder exhibiting the rotatory power a_D 173.3° to 174.1°. The results of the ultimate analysis indicate the formula $C_6H_{10}O_5$. On hydrolysis with dilute acids, practically the whole is converted into *d*-glucose. Iodine in potassium iodide gives a powerful brown-red stain, on which account the substance has received the name **erythrocellulose**. It differs from the somewhat analogous glycogens in its lower rotatory power. The other constituent of the original residue remains in the form of a colourless jelly at the bottom of the liquid, and, as it is not altered by iodine solution, is called **achroocellulose**. On hydrolysis it furnishes a mixture, chiefly of glucose with comparatively little mannose, and on this account is probably not a uniform substance.

From the above reports one thing seems clear: that the membrane of the yeast cell is a complex structure, both from the anatomical and the chemical point of view. This fact will be regarded as so much the more certain when it is stated that no one has yet succeeded in obtaining the so-called yeast cellulose free from ash constituents and nitrogen. Schlossberger found 1.0 per cent. of ash, and the preparations made by Salkowski contained between 1.7 and 2.6 per cent., whilst Liebermann and Bitto (I.) found 1.8 per cent. in theirs. It does not do, however, to launch out into theories respecting the significance of these inorganic constituents, the more so because the composition of this ash is still unknown. Moreover, we have also to reckon with the still uninvestigated possibility that the ash itself is introduced into the membrane during the process of extraction. As regards the amount of nitrogen in the membrane, one is unable to find similar unanimity in the reports of the different workers, for whereas Schlossberger (I.) could not reduce it below 0.5 per cent. and E. Salkowski (VI.) arrived at about the same limit (0.4 to 0.5 per cent.), both Mulder, and also Nägeli and

Löw (II.) claimed to have eliminated it to a trace, though by the use of reagents (*e.g.*, strong, warm hydrochloric acid) which few substances could withstand undecomposed.

§ 250.—The Cell Nucleus.

Only in comparatively rare instances is the **cell nucleus** easily recognisable in the living cell without some preliminary treatment, its refractive power being usually only a little greater than that of the **cytoplasm.** In years gone by, when the optical instruments available were inferior in power to those of the present day, the nucleus thus escaped the observation of even practised microscopists. Whereas Nägeli, as long ago as 1844, frequently remarked in the cells of wine and beer yeasts "a small nucleus of whitish mucus adjoining the membrane," the presence of which was confirmed by M. J. Schleiden (I.) in 1849, Ernst Brücke (I.) on the other hand asserted in 1861 that he had never succeeded in finding the same. The earliest important advance was made towards the close of the eighties, by the application, to the botanical examination of cells, of the microchemical staining processes already successfully used in the domain of zoology. Fr. Schmitz (I.) in 1879 prepared yeast cells by hardening them in a saturated solution of picric acid, then washed them with care, and stained them by immersion in hæmatoxylin, followed by repeated washing. In this way he succeeded in discovering in each cell a small blue-stained body, which was clearly distinguishable from the surrounding colourless plasma, and, both in this and several other respects, behaved very like the nucleus of the animal cell. Strasburger, in repeating the tests several years later, descanted on the somewhat difficult performance of this staining process, and it is therefore not surprising that other experimenters of both sexes, *e.g.*, Fr. Krassner (I.) in 1885, and Sidonia Eisenschitz (I.) ten years later, tried it without success. No more fortunate in this respect was Joh. Raum (II.), who, in 1890, on the basis of his staining tests (mentioned in later paragraphs) expressed the opinion that the cells of yeast are non-nucleated. A similar negative standpoint seems to have been taken up also by G. Hieronymus (I.) and A. B. Macallum (I.). On the other hand, Schmitz's discovery was confirmed by E. Chr. Hansen (XVI.), A. Zalewski (I.), E. Zacharias (I.), H. Möller (II.), P. A. Dangeard (II.), Fr. A. Janssens (I.), and L. Buscalioni (I.). The first-named Danish worker also stated that he had been able to discern the nuclei in the cells of the films produced by *Saccharomyces Pastorianus I.* and *II.*, and *S. ellipsoideus I.*, in many cases without trouble. Möller also observed the nucleus as a homogeneous, pale reddish disc, and expresses the opinion that the same has been mistaken for a **vacuole** by many observers. The present author is able to confirm this from his own experience as a teacher, this mistake regarding the cell nucleus being of somewhat frequent occurrence among practitioners.

The method tested by H. Möller (III.), and recommended by him as the best for rendering the nucleus visible, is a staining process proposed by H. Heidenhain (I.); and Janssens and Leblanc (I.) also recommended the same as being the most reliable. The method of application consists in fixing a cover-glass streak-preparation with a solution of iodine in potassium iodide, and hardening the same in alcohol as recommended by Möller (II.); then mordanting it in a $2\frac{1}{2}$ per cent. solution of iron alum, and finally staining with a $\frac{1}{2}$ per cent. solution of hæmatoxylin.

The morphological variability of the cell nucleus was already recognised by Schmitz. In young cells the nucleus is **globular**, but flattens with increasing age to a disc of sinuous contour (Zalewski found this elliptical). The dimensions are considerable, the diameter in some instances attaining to one-third that of the

cell itself. According to the researches of Buscalioni and Casagrandi (I.) with *Saccharomyces guttulatus*, the dimensions of the nucleus vary with the size of the cells. Except in the case of ascosporulation (to be referred to later on), Janssens and Leblanc (I.) failed to find more than a single nucleus in any yeast cell during their experiments with pure culture.

The first observation in connection with the internal structure of the yeast-cell nucleus, namely, the occurrence of a **nucleolus**, was made by Zalewski (I.) in 1885. H. Möller (II.) in 1892 questioned the accuracy of this report, but it was confirmed a year later, almost simultaneously, by Dangeard (II.) and Janssens (I.), and also later by H. Wager (I.) who found that the cell nuclei of yeast exhibit both a membrane and a nucleolus. The latter is of globular shape, is situated about the centre of the nucleus, and measures in diameter up to one-third that of the last-named. The space between the membrane and the nucleolus is filled with **karyoplasm**, which, according to the researches of Janssens and Leblanc (confirmed by H. Wager) has a filamentous structure, forming a sort of framework. The nucleolus is suspended by the threads of karyoplasm. These two Belgian workers also observed that, under certain circumstances, one or more vacuoles may appear in most of Hansen's species of *Saccharomycetes*, as also in *Schizosaccharomyces Pombe* and divers beer yeasts and pressed yeasts, especially when the cells are transferred into a fresh nutrient solution. They have also been observed by Buscalioni in the nuclei of *S. guttulatus*, but are never found in *Sacch. Ludwigii* and *Schizosaccharomyces octosporus*.

A few remarks may here be made as to the division of the nucleus. The reports hitherto made on the form and structure of the nucleus of yeast have always related to cells not actually engaged in reproduction. When, however, this process sets in, a similar division (reproduction) of the hitherto quiescent nucleus also begins. This operation, which has been already mentioned in § 46 and § 219, may proceed in two ways: either the comparatively simple process of direct subdivision, known as **fragmentation** or **amitosis**, or by the indirect division termed **segmentation**, **karyokinesis**, or **mitosis**. In this latter method the two daughter-nuclei are the result of extensive alterations and modifications of the nuclear structure, as may be read in detail in the treatise of A. Zimmermann (II.) already mentioned. So far as yeast cells are concerned, a certain lack of unanimity on this point prevailed for some time among the various observers. Thus the nuclear reproduction accompanying gemmation was regarded as fragmentation by H. Möller (II.) in 1892, and this opinion was shared by Dangeard (II.) a year later, Buscalioni (I.) taking the same view in 1896, and H. Wager (I.) in 1898. On the other hand, Janssens (I.) in 1893 opined that the nucleus in the budding yeast cell reproduces by karyokinesis. Equally contradictory are the reports on nuclear subdivision during the formation of ascospores. Janssens considered it karyokinetic, but Möller (IV.) and Buscalioni held it to be fragmentation. The problem was solved in 1898 by the observation of Janssens and Leblanc (I.) that the nuclei of yeast cells, especally *Saccharomyces Ludwigii* and *Schizosaccharomyces octosporus*, are usually reproduced by karyokinesis, but that this process may undergo a more or less extensive simplification and approximation to direct division, more particularly when the cells are kept under comparatively less favourable conditions of existence. Similar intermediate stages between the two extremes, of mitose on the one hand and amitose on the other, have also been recorded in respect of the cell nuclei of higher plants during the past few years.

We are also indebted to the two last-named Belgian workers for a series of observations on the progress of nuclear subdivision. The first thing noticeable in a cell that is about to bud is the disappearance of the nuclear membrane, and the elongation and separation of the nuclear body into two parts. The two

nucleoli, which are still connected by a thin thread, then move towards that part of the cell at which the bud commences to protrude. One of them passes into the embryo daughter-cell, and develops into the nucleus thereof, enveloping itself with a membrane—as does also the other which has remained in the parent-cell. A view of the different stages of this subdivision is given in Fig. 150. During sporulation a similar subdivision occurs. Two daughter-nuclei are first formed, which, of course, remain in one and the same cell, and thereby give rise to the exceptional case referred to above, of more than one nucleus being present in a yeast cell. Both these nucleoli eventually undergo a second subdivision, the resulting four nuclei then consuming the existing cytoplasm for the purpose of developing into a corresponding number of ascospores, each enveloped in a new membrane. In the event of eight spores being formed, the process of subdivision must first be repeated. In cells containing a different number of ascospores (*e.g.*, six or nine), it naturally follows that the process of subdivision has not been repeated the same number of times in all the nuclei, but has ceased in some earlier than in others. Finally, it may be mentioned that the processes just referred to cannot be distinguished in the living cell without preparation—as Krasser (II.) unsuccessfully endeavoured to accomplish—but that recourse must be had to a suitable method of staining in order to render them visible.

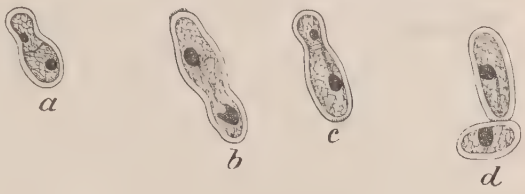


Fig. 150.—Saccharomyces Ludwigii, Hansen.

Cells at various stages of budding. Stained.

a, Subdivision of nucleus accomplished; the daughter-nucleus entered into the daughter-cell still remains attached to the connecting thread;

b, the latter becomes detached;

c, the septum between mother and daughter cells commences to form;

d, the separation is complete. Magn. 1200. (*After Janssens and Leblanc.*)

Since, as already stated, the nucleus in an unstained yeast preparation may readily be mistaken for a vacuole, and *vice versâ*, by an unskilled eye, it is necessary to make a few remarks with regard to these vacuoles. As a rule—to which an exception is afforded by, *e.g.*, *Saccharomyces apiculatus*—the appearance of one or more vacuoles in the yeast cell is coincident with exhaustion and with a lack of nourishment. Up to the present no reliable information is available as to the nature of the liquid composing these vacuoles. The refractive power is lower than that of the surrounding plasma, which circumstance alone is sufficient to differentiate them from the ascospores with which they are occasionally confounded by beginners, since a closer examination will quickly show that the refractive power of the latter bodies is (in most cases at least) greater than that of ordinary yeast plasma. This difference becomes particularly apparent when the micrometer screw of the microscope is turned to and fro a little. Under certain circumstances the development of vacuoles (which are mostly globular or oval in shape) may proceed so far that they almost completely fill the cells, the residue of the protoplasm being then reduced to a thin stratum adjacent to the cell walls; or, where two or more vacuoles are present, may form a thin layer between them,

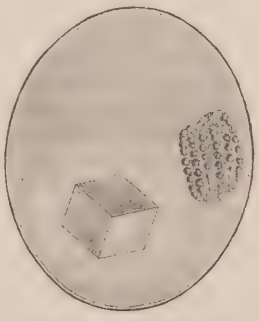


Fig. 151.—Crystalloid in the vacuole of a cell of pressed yeast. Near by is a dense cluster of granules. Magn. 4400.

(*After Hieronymus.*)

and thus, to the inexperienced eye, produce the erroneous impression that a septum has been developed.

The vacuoles are also occasionally observed to contain **inclusions**. An instance (though a comparatively rare one) of this kind, namely, the appearance of crystals or crystalloid structures, is shown in Fig. 151. Such inclusions have been observed by Raum (II.) and Hieronymus (I.) in the vacuoles of true *Saccharomycetes*, and by H. Will in those of *Mycoderma* and *Torula*. Janssens and Leblanc, on the contrary, are of opinion that these structures are in reality nucleoli.

A more frequently observed occurrence is the presence, in vacuoles, of a briskly motile body, of which mention will be made at the end of the next paragraph.

§ 251.—The Granules.

In addition to the nucleus, which, as a rule, is not visible until the preparation has been stained, yeast cells generally contain other organised inclusions, which, on the contrary, do not require any artificial aid to become visible, but compel the attention of the observer by their higher refractive power. Since their lustre recalls that of the drops of oil found in the cells of higher plants, *e.g.*,

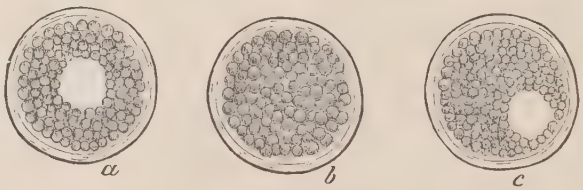


Fig. 152.—Permanent Cells

of bottom-fermentation beer yeast, either partially or entirely filled with granules. Cell wall considerably thickened. Magn. 2000. (*After Will.*)

in seeds, they have received the name of "**refractive bodies**," or, formerly, "**oil drops**." At present they are known as **granules** or **microsomata**. It is only in very young cells that they are not to be found; but they appear in large numbers at the close of primary fermentation, and are usually very abundant in permanent cells (§ 245). Naturally the method of nutrition has also some influence on their occurrence. Thus G. Hieronymus (I.) found only very few granules in the cells of pressed yeast that had been grown in a nutrient-salt solution containing grape sugar, whereas they were abundant in such cells as had been kept in milk or a solution of beet sugar. According to a report by Joh. Raum (II.), no granules are formed when sugar is absent from the nutrient substratum.

It is to the last-named worker that we are indebted for the first accurate investigations in connection with these inclusions. These researches are the more valuable in that they were exclusively performed on pure cultures, namely, of ten species of budding fungi, comprising six true *Saccharomycetes* (*S. cerevisiæ I.*, Hansen, *S. ellipsoideus I.* and *II.*, H., *S. Pastorianus I.*, H., pressed yeast from Warsaw, and a yeast from the air), a red budding fungus termed *S. glutinus*, a so-called black yeast, and a budding fungus from Kephir granules. The occurrence of the inclusions in question was demonstrated in all these species. The number fluctuates considerably, and very often far exceeds a dozen. Their dimensions were also found to vary considerably. When a large number are present, they are comparatively minute (Fig. 152), generally only a few tenths of a micromillimetre in diameter; but when the number is smaller (and it may

sink to unity), the size attains considerable proportions. In the latter event they may readily be mistaken, by the inexperienced eye, for ascospores, owing to the high refractive power they have in common with the latter. In doubtful cases the uncertainty may be removed by the aid of some of the microchemical reactions given below. Moreover, these granules are not always of the same shape; for the most part they are globular, though specimens with an angular contour are by no means infrequent. In throwing doubt on the existence of this latter modification, and opining that it only appears on staining, JANSSENS and LEBLANC (1.) forget that it has also been observed by Hieronymus in the granules of unstained, living yeast cells.

H. WILL (VIII.) was the first to publish details concerning the anatomical structure of these granules. Whereas J. Raum had previously failed to detect any structure, and Hieronymus regarded them as crystalloids, Will succeeded in showing, with a series of pure cultures of beer yeasts of divers origin—*Mycoderma*, *Torula*, and species belonging to the *Saccharomyces anomalus* group—that the granules in question consist of at least two distinct constituents: an outer case of albuminous substances and a fat-like interior, which latter justifies the name "oil drops" bestowed by Will. The only point on which doubt exists is with regard to the presence of filaments (Fig. 153) which, according to the observations of this worker, extend from the outer case to the interior of the granule and there form a network enclosing the fatty contents of the granule. O. CASAGRANDI (I.) regards this network as being, not a peculiarity of the unaltered granulum, but as a consequence of the influence of the reagents (*e.g.*, alcohol) employed for dissolving out the fatty portions and revealing the alleged reticulations. For the rest, and in its chief points, Will's observation was confirmed by Casagrandi.

FIG. 153.—Permanent Cell of bottom-fermentation beer yeast, containing an unusually large granule. After treatment with alcohol. Magn. 2000. (*After Will.*)

The peculiar behaviour exhibited by the granules towards reagents is easy to understand. With the exception of RAUM and FR. KRASSER (II.) all the other observers—including Alb. Klöcker in the case of the so-called *Saccharomyces apiculatus*—are entirely agreed that the granules are turned brown by the action of a 1 per cent. solution of perosmic acid, which is the chief reagent for fats. The fatty contents having been extracted by a suitable solvent, the residual outer case gives all the characteristic reactions for albumen. When yeast cells are treated with concentrated sulphuric acid the membrane swells up; the acid then gains access to the granules and destroys their integument, whereupon the fatty contents of the individual granules coalesce to form larger drops. These turn first green and then bluish green to blue-black, and, on the addition of a little 10 per cent. caustic potash, are partly or entirely dissolved, *i.e.*, vanish from sight. According to Casagrandi's confirmation of the results obtained by Raum, a similar effect is obtained by treating yeast cells with artificial gastric juice: the outer case of the individual granules is digested, whereupon the exposed contents unite to drops, which can then be removed by ether-alcohol.

The action of fat-dissolving reagents on the contents of the still unaltered granules is, as may well be imagined, considerably impeded by the albuminous integument, and requires some time (often far more than twenty-four hours) to attain completion. Lacking the necessary patience for this, one is easily led to the conclusion that the solvent has no influence; as was the case, for instance, with Raum and the ether-alcohol mixture, with Hieronymus and concentrated caustic potash, and with CURTIS (I.) and benzene, until Casagrandi, with a greater exercise of patience, showed the contrary. Moreover, it is not surprising that

the requisite duration of exposure to these reagents should have been found to vary, not only with the granules of different cells, but also in the case of the different granules in one and the same cell. It is, however, carrying matters to extremes to do as was proposed by Sidonia Eisenschitz (I.), for example, and attempt to establish a classification of the granules into different groups on the basis of this divergent behaviour.

Fig. 154.—Dead Permanent Cells from bottom-fermentation beer yeast; each containing a large granule which has burst in consequence of the application of pressure to the cover-glass. Magn. 2000. (*After Will.*

From the above-mentioned solubility of the outer cases of the granules in artificial gastric juice, it may be concluded that they are constructed of digestible albuminoids, and consequently that no nuclein is present in the granules. Nevertheless, according to the reports of Fr. Krasser (II.), this rule, which was established by E. Zacharias (I.), has certain exceptions. With regard to the composition of the fat-like contents of the granules, nothing precise can be said at present. Attention should, however, be paid to the reports recorded in the third paragraph from the end of § 253.

The observations (especially those of H. Will) made on the consistence of the granules, show that the fatty contents of these inclusions are not perfectly liquid, but rather semi-fluid. The application of pressure to the cover-glass, under which a yeast cell has been placed, causes the granule to burst, as is shown in two examples in Fig. 154.

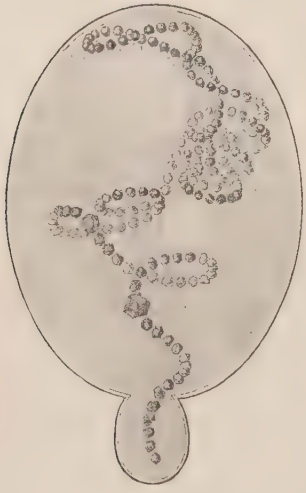


Fig. 155.—Granules in a living cell of pressed yeast in course of budding. Several of the granules are decidedly multiangular, two of them being also much larger than the rest. The two white areas represent vacuoles. Magn. 4400. (*After Hieronymus.*)

With regard to the distribution of the granules within the yeast cell, it was found by J. Raum that a certain order prevails, inasmuch as these structures are arranged in series which represent arcs or portions of arcs. Closer investigations, conducted by Hieronymus, supplemented this discovery by showing that the granules are arranged in spirally wound chains, embedded in a continuous protoplasmic filament as depicted in Fig. 155. Hieronymus calls this union a central filament. Subsequently, on the basis of the observations of A. Goertz, the existence of such an arrangement of the granules was denied by A. Zimmermann (IV.), until Casagrandi confirmed the accuracy of Hieronymus' discovery, with the unimportant limitation that the alleged regularity of arrangement is, in many cases, present to only an inferior extent.

Clearly under the influence of Bütschli's theory of the structure of protoplasm (p. 33, vol. i.), Janssens and Leblanc (I.) explained the appearances in question as the knots of the network observed by them in the cytoplasm of the yeast cell (Fig. 156). In the author's opinion, however, it has not yet been strictly proved that these knots are identical with the structures here referred to as granules. The existence of a reticulated framework in the cytoplasm of the yeast cell has, nevertheless (according to a written communication), been also demonstrated by H. Will.

Staining the granules for the purpose of identification can be effected by the method recommended by P. Ernst (II.) and tested by Raum. The preparation is treated with moderately warm (not boiling hot) Löffler's Methylene Blue solution, followed by washing with water, and finally by staining with a cold solution of Bismarck Brown. This causes the granules to appear black, and to stand well out from the surrounding brown-stained plasma. A differential staining of the integument of the granules can be effected by the method recommended by Casagrandi. Here the yeast cells are fixed (§ 34) with an alcoholic solution of corrosive sublimate, the fatty contents of the granules are then extracted with absolute alcohol, the rest stained with a 20 per cent. solution of fuchsine, decolorised, and finally stained by immersion in a 30 per cent. aqueous solution of picric acid. This colours the integument red and causes the granules to stand out clearly from the surrounding plasma, which is stained yellow. Even during the life of the cell, the granules will absorb certain dyestuffs, for instance when, as recommended by Eisenschitz, cells of yeast are cultivated in beer wort containing an addition of a 1 per cent. solution of Benzopurpurin. Some of the granules will stain red by this method in one to two days.

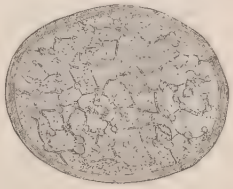


Fig. 156.—Belgian Beer Yeast after a sojourn of forty-four hours in beer wort; observed in the live state. Exhibits the plasmal framework, with appurtenant knots, referred to in the text. The cell nucleus is not depicted. Magn. 2000. (*After Janssens and Leblanc.*)

In addition to these granules, which can be detected at once in the unchanged living cell, there are, according to Hieronymus, other similar inclusions, which only become visible after the cells have been fixed with, say, chromic acid or hydrochloric acid. When this treatment has been followed by staining, the "**central filament**" is found to be surrounded by an abundantly looped chain of granules embedded in the portions of the plasma adjoining the wall.

Nearly every one of the workers hitherto named has recorded a certain power of **locomotion** on the part of the structures in question, though the reports on the extent of this power differ. It was remarked by Raum, and then confirmed by Hieronymus, that in many instances (though not invariably) a gradual migration of granules from the parent-cell into the young bud goes on during the formation of a daughter-cell. This is represented in Fig. 155. The movement of the granules, however, proceeds very slowly, and bears no comparison with the brisk saltatory motion (Brownian movement?) noticeable in the bodies often observed in the vacuoles of many yeasts, and which are indeed so plentiful in the cells of *Mycoderma* as to constitute a characteristic of this mould fungus. Divergent opinions prevail on the nature of these vacuolous inclusions (so-called saltatory bodies), some regarding them as being invading bacteria, others as endogenous cells, or as dead plasmal excretions. J. Raum was the first to observe the entry of these bodies from the plasma into the vacuole; and it was then shown by E. Kuester (I.) that this migration could be induced by allowing the yeast cells to dry on some substratum. According to the reports of Eisenschitz, a reversal of this migration—namely, from the vacuole back to the surrounding cell plasma, may occasionally take place. The chemical construction of these saltatory bodies can no longer be regarded as identical with that of the plasma granules, since E. Kuester has shown that the two differ in certain respects as regards their behaviour towards dyestuffs. This notwithstanding, neither he nor Symmers (II.) later on, succeeded in arriving at a definite solution of the problem.

In many instances it is observed that the granules offer a greater resistance

to decomposition than any other constituents of the yeast cell, so that, even when the cell membrane has become warped by moisture and the other portions of the cell contents have disappeared from view, the granules remain alone, in the form of free globules (§ 8) as fine as dust, which swim in the nutrient medium, and occasionally render the latter turbid. Probably it is in this sense that we should regard the observations which J. Wortmann (XII.) had occasion to make in connection with wines that had turned turbid during storage in bottle.

CHAPTER XLVIII.

CHEMISTRY OF THE YEAST CELL.

§ 252.—Chemistry of the Yeast-Cell Nucleus.

Recent investigations have shown the probability that, in place of being in a free state in the cell, the **albuminoids** present form part of more highly constituted bodies termed **proteids**. At a given moment these bodies are split up into the albuminous nucleus on the one hand, and the adherent lateral chains on the other. These latter have been termed prosthetic groups by A. Kossel (V.), and explained as the real means by which the vital activity of the cell is enabled to act. Of the groups into which the proteids themselves have been classified, one, namely that of the **nucleins**, is of particular importance, since, as the name itself implies, it furnishes important structural material for the nucleus. A little fuller information on this point will probably be not unwelcome to the reader, and for this reason a few remarks concerning certain conclusions of a practical nature will now be given.

In 1869 F. Miescher (I.) isolated, from the nuclei of pus cells, a nitrogenous constituent to which he gave the name **nuclein**. This body differed from other proteids, both as regards its percentage of phosphorus and by its power of withstanding pepsin. The occurrence of such nuclein in yeast cells was then discovered by F. Hoppe-Seyler (IV.). After doubt had been cast by Nägeli and Löw (II). on the accuracy of these observations, A. Kossel (III.) succeeded in obtaining considerable quantities of fairly pure nuclein from pressed yeast. For this purpose the yeast, stirred to a pulp, was left for several hours under water, the latter being renewed once or twice in the interim. The yeast sediment was then introduced into dilute caustic soda, which extracted the nuclein. As, however, the latter was at the same time gradually attacked by the solvent, it was found desirable to place the mixture at once on a number of filters and to allow the filtrate to drop into dilute hydrochloric acid, which re-precipitated the dissolved nuclein. The precipitates were then united on a filter, washed with dilute hydrochloric acid and alcohol, and repeatedly extracted with the same, thus furnishing a product which, when dried under the air-pump, formed a white and fairly pure preparation. Analogous substances have been separated from the most divergent animal and vegetable organs, and classified under the collective term nucleins.

The first investigations concerning the percentage proportion of nuclein to the total nitrogenous constituents of yeast were performed by A. Stutzer (I.). According to this worker, the dried residue obtained from beer yeast by several days' cold extraction with 95 per cent. alcohol, followed by drying over sulphuric acid, contained 8.65 per cent. of total nitrogen, of which 2.26 per cent. (or more than a quarter) was in the form of nuclein. Still richer in this respect was the thallus of a mould fungus of undetermined species, which settled from the air into a nutrient solution containing tartaric acid; since, out of 3.78 per cent. of total nitrogen in the dry residue, 1.54 per cent. (or nearly 41 per cent. of the whole) was present as nuclein.

It is to the labours of A. Kossel and his pupils that we are mainly indebted

for information on the chemical constitution of nuclein in general, and of yeast nuclein in particular. Their discoveries furnished the basis for Kossel's (II.) classification of the nucleins into two groups. The first includes substances which, so far as is known, do not occur in cell nuclei, and which were named **paranucleins** by Kossel, or **pseudonucleins** by Hammarsten (III.). When decomposed by dilute acids they yield only phosphoric acid and albumen. On the other hand, under the same treatment, the true nucleins, which alone form the subject of the following lines, furnish, in addition, basic substances to which the name nuclein bases has been given.

An important elucidation of the constitution of nuclein was first presented by R. Altmann (I.) by the discovery that these proteids are decomposed by dilute alkalis into albumen and a nitrogenous acid, rich in phosphorus, which has received the name of **nucleic acid**. This therefore is the prosthetic side chain in the nuclein complex; and it generally receives an additional appellation indicative of the origin of the nuclein in question. All the varieties of nucleic acid exhibit the characteristic behaviour, when heated with dilute acids, that they suffer decomposition into one or other of the nuclein bases described below. The molecule of nucleic acid is composed of a large number of atoms. Those of phosphorus and nitrogen stand in the ratio 1 : 3 in nearly all the instances known. So far, only two exceptions to this rule have been observed. One of them is the **guanylic acid**, isolated from the nuclein proteid of the pancreas of the ox and more closely examined by I. Bang (I.), the molecule of which acid contains 5 atoms of nitrogen to each atom of phosphorus. The other is a nucleic acid isolated from the embryo of wheat by Th. B. Osborne and G. F. Campbell (I.), the ratio P : N being in this case 1 : 4.

As regards the composition of the albuminoids resulting from the decomposition of the nucleins, less is known than of the nucleic acids with which they are combined. A. Kossel (III.) was the first to investigate yeast nuclein, and he found that the albuminoid substance obtainable therefrom offered greater resistance to the digestive enzymes (pepsin and trypsin) than the yeast nuclein itself, since about 66 per cent. of the latter, but only 3 per cent. of the former, could be brought into solution within twelve hours in the course of a comparative test.

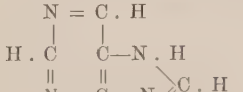
According to A. Kossel (II.), the yeast nucleic acid, first prepared by Altmann, has the formula $C_{17}H_{26}N_6P_2O_{14}$ or $C_{25}H_{36}N_9P_3O_{22}$. The same authority (V.) states that it is decomposed by alkalis into carbohydrates and an acid, namely, **plasmic acid**, rich in phosphorus and in nitrogen. The formula ($C_{15}H_{28}N_6P_6O_{30}$) ascribed to this latter acid by Kossel is apparently only approximately correct, since no notice was taken of the iron content (p. 35, vol. ii.) subsequently found therein by A. Ascoli (I.). Under the influence of boiling dilute mineral acids, plasmic acid furnishes nuclein bases, together with phosphoric acid and a nitrogenous organic substance which has not yet been more closely examined. A patent has been taken out by K. Schwickerath (I.) for a process of purifying crude nucleic acid from adherent brown mucinous substances, in view of the preparation of yeast nucleic acid on a large scale. In this connection, attention should also be directed to the German Patent No. 107,734, granted to the Elberfeld Farbenfabriken.

The carbohydrates furnished by the action of boiling dilute mineral acids on yeast nucleic acid (though not from plasmic acid) reduce Fehling's solution, and, according to Kossel (V.), probably consist of a mixture of glucose and one of the pentoses. Liebermann and Bitto (I.) regarded them as an adherent contaminating admixture, of the character of the so-called yeast gum. Similar carbohydrates have also been isolated from various nucleins of animal origin by A. Kossel and A. Neumann (II.), as also by Hammarsten (III.), Alfr. Noll (I.), and by I. Bang (I.). The two first-named workers, however, have also shown that many

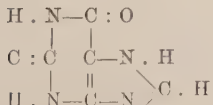
nucleins contain two kinds of carbohydrates: non-reducing carbohydrates present in the molecule of the nucleic acid; and reducing carbohydrates which, along with the latter substance, take part in the structure of the nuclein complex, and appear in company with nucleic acid on the decomposition of the main substance.

Of the nuclein bases here in question, the first to be discovered in the manner described above was **hypoxanthin** ($C_5H_4N_4O$), which was obtained from yeast nuclein by Kossel (III.) in 1879. Two years later he prepared 10 grams of this base direct from pressed yeast by a method (VII.) of his own, which he afterwards improved (IX.), and showed (VIII.) that the same is also obtainable from nucleins of different (animal) origin. In the meantime he isolated (III.) from yeast nuclein a second base, **xanthin** ($C_5H_4N_4O_2$), to which he added a third, namely, **guanin** ($C_5H_5N_5O$) in 1882 (IX.). The occurrence of the last-named in yeast has also been placed beyond doubt by S. Schindler (I.). These three allies of uric acid ($C_5H_4N_4O_3$) had already been long known to chemists, though not as constituents of nucleins. On the other hand, the four additional bases mentioned below were all new and were obtained for the first time from the nucleins. **Adenin** ($C_5H_5N_5$) was isolated by Kossel (XI.) in 1885, first from the nuclein of ox pancreas and afterwards from pressed yeast. This substance stands in the same relation towards hypoxanthin as guanin does to xanthin, not only as far as chemical composition is concerned, but also in respect of behaviour during putrefaction caused by certain bacteria, which, according to the researches of S. Schindler (I.), degrade guanin to xanthin, and adenin to hypoxanthin. According to A. Baginsky (IV.), the last-named offers the greater resistance to the influences in question. In 1893 another new base, namely, **thymin**, was brought to our knowledge by A. Kossel and A. Neumann (I.). It was first obtained from the nucleic acid isolated from the thymoid gland of the calf, which acid received the name of **adenylic acid** on account of its capacity for furnishing adenin. The thymin molecule (which has the formula $C_5H_6N_2O_2$) may be regarded as methyl-dioxy-pyrimidin, in conformity with the views of H. Steudel (I.), or as 5-methyl-uracyl, in sympathy with those of E. Fischer and G. Röder (I.), who were the first to prepare it by synthetic means. The hypothesis raised by Kossel and Neumann (II.) that thymin is also present in the molecular complex of the nucleic acid of yeast, has not been confirmed, though, according to A. Ascoli (III.), the parent substance of thymin, namely, **uracyl** ($C_4H_4N_2O_2$) is found therein. It may also be remarked by the way that the fourth of the new nuclein bases, **cytosin**, has so far only been found as a constituent of nucleic acids of animal origin. In the crystalline state this substance has the formula $C_{21}H_{30}N_{16}O_4 . 5H_2O$.

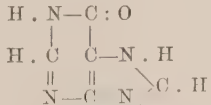
Apart from the last-named, which has not yet been sufficiently investigated, the nuclein bases mentioned may be classified into two groups in accordance with their constitution. Adenin, xanthin, hypoxanthin, and guanin are derived from the atomic complex to which Emil Fischer (I) gave the name **Purin**:



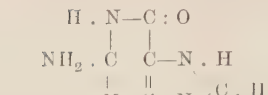
Purin.



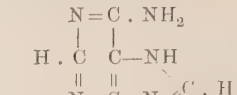
Xanthin.
$C_5H_4N_4O_2$.
2-6-dioxy-purin,



Hypoxanthin.
$C_5H_4N_4O$.
6-monoxy-purin,

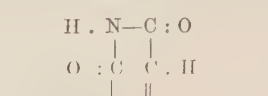


Guanin. $C_5H_5N_5O$.
2-amino-6-oxy-purin.

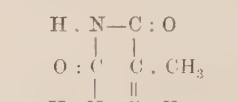


Adenin. $C_5H_5N_5$.
6-amino-purin.

They accordingly belong to the large group of purin bases. On the other hand, thymin and Ascoli's yeast nuclein base are of more simple construction:



Ascoli's base. $C_4H_4N_2O_2$.
Uracyl.



Thymin. $C_5H_6N_2O_2$.
5-methyl-uracyl.

Of these, thymin seems the most widely diffused, since only three nucleins not containing this base are known, one of them being yeast nucleic acid. The second is the above-mentioned guanylic acid, which, according to I. Bang (I.), furnishes guanin alone (and of this about 35 per cent. by weight). The third is **inosinic acid**, which was discovered by Liebig in meat juice, and which, according to the experiments made by F. Hasier in 1895, probably contains hypoxanthin solely.

The percentage content of xanthin, guanin, hypoxanthin, and adenin (of course in the combined condition specified) in yeast, was first ascertained by S. Schindler (I.) by the aid of his quantitative method of separation, and was found to be 0.024, 0.029, 0.093, and 0.043 gram respectively per 100 grams of pressed yeast, the character of which was not more accurately defined. These values should not be considered as more than merely approximate, the reliability of analytical methods having been proved doubtful by C. Wulf (I.).

To the reader the situation of nuclein in cell nuclei generally, and in those of the yeast cell in particular, will not appear to have been clearly defined in the foregoing lines. This view is correct. By macrochemical means it is impossible to arrive at more than a hypothesis possessing a certain probability, it being found that cells or agglomerations of cells containing either a large number of nuclei or nuclei of considerable size, yield a higher proportion of nuclein than such as contain only a few nuclei or those of small dimensions. This was the conclusion formed by Miescher, who succeeded in separating the cell nuclei from the pus cells on which his exhaustive experiments were performed. By similar comparative methods, A. Kossel (I.) came to the conclusion that nuclein, instead of being a reserve material, plays an active part wherever new cells are in course of formation and nuclear reproduction is consequently in progress, *i.e.*, in all phenomena of germination. Thus the formation of nuclei and nuclein proceeds simultaneously. This opinion was also shared by H. A. Landwehr (I.). The sole method of obtaining reliable information with regard to the situation of the nuclein within the cell is by microchemical examination. With this object use can be made of the high resistance offered by the nucleins towards pepsin. For example, E. Zacharias (I.) detected the presence of nuclein in the nuclei of pressed yeast cells by the aid of so-called artificial gastric juice (a solution of pepsin in 0.2 per cent. hydrochloric acid). The staining methods, however, are capable of more extensive application.

The various individual chemical constituents of the cell exhibit divergent

absorptive affinities for different dyestuffs. It is known that from a mixture of red, blue, or green dyestuffs one cell constituent will absorb chiefly or entirely the first-named, *i.e.*, erythrophil, whereas another constituent will prefer the blue (or green), and is therefore termed cyanophil. This preference, however, in each case is determined, not by the shade of colour, but by the chemical constitution (or reaction) of the dyestuff on the one hand, and by that of the cell constituents with which it is brought into contact on the other. A cell constituent is therefore said to be **acidophil** when, from a mixture of acid and basic dyestuffs, it absorbs the former and becomes stained therewith; in the converse case it is termed **basophil**. Chemical affinity is almost the sole factor influencing the occurrence or non-occurrence of staining, so that, in presence of a mixture of an acid red dyestuff and a basic blue dyestuff, a given basophil cell constituent will behave as a cyanophil, but, in presence of a mixture of a basic red and an acid blue dyestuff, as an erythrophil. An instance of this kind is afforded by the nucleic acid of yeast, which is strongly basophil, and, according to observations made by H. Malfatti (I.), E. Zacharias (II.), and L. Lilienfeld (I.), is stained (blue or green) by a mixture of Acid Fuchsine and Methyl Blue (or Methyl Green), but red by a mixture of Safranine and Pale Green. Such behaviour having been macrochemically observed with the known dyestuffs, the same can also be employed microchemically for examining the cell for the presence of one or another chemical substance occurring in local accumulations therein. In any event, care is necessary in the preliminary treatment of the preparation, since in many cases this has a decisive influence on the results. For more detailed information on this point the reader is referred to the monograph by A. Zimmermann (II.). In so far as these microchemical reactions enable a reliable decision to be formed, it has been shown in this manner that the nucleus of the yeast cell is rich in nuclein, and that the latter is probably not present in the cytoplasm. Now, as already shown in § 250, the nucleus consists of the reticulated framework, the sap (or juice), the membrane, and the nucleoli. The first-named, in turn, is composed of the reticulated matter known as **linin**, which is difficult to stain, and of the globules situated at the intersections of the network, these globules consisting of a substance which is readily penetrated by dyestuffs, and which has therefore received the name **chromatin** (§ 35). The nuclein is located in this chromatin, whereas the filaments of linin and the nucleoli contain, in addition to albumen, a substance termed **plastin**, which differs appreciably from, although closely allied to, the nucleins.

The question as to the amount and nature of the other nitrogenous substances associated with the nucleoproteids in the nucleus of the yeast cell cannot at present be satisfactorily decided, the reports on this matter being few in number and not reliable. The same applies to the nitrogenous constituents of the cytoplasm (§ 219), the scanty communications referring to which will be dealt with in § 255.

The composition of the yeast water prepared in the manner described in § 82 will be briefly considered here from the standpoint of the reports mentioned in the foregoing paragraphs. Even mere boiling in water is sufficient to break up the yeast nuclein, with formation of nuclein bases and free phosphoric acid, which therefore occur as constituents of yeast water. The appearance of phosphoric acid in decoctions of yeast had already been observed by A. Béchamp (VII.), who found, in 1865, that 100 grams of dry yeast lost, on extraction, 2.8 to 3 grams of phosphoric acid, part of which was in the free state. This was confirmed by A. Kossel (III.). He also found that this liberation proceeds rapidly at first, but later on very slowly. With regard to the extraction of nitrogenous substances (xanthin, guanin, sarcin, carnin, leucin, and tyrosin) from yeast cells by the water

in which they have been boiled, certain experiments were made in 1874 by P. Schützenberger (I.), who, by repeatedly extracting yeast with boiling water until exhausted, found that, of the 2.78 grams of nitrogen present in 100 grams of fresh yeast (containing 30 per cent. of dry matter), 0.75 gram could be extracted in this manner. In distilling fermented distillery mash, a larger or smaller amount of the nuclein of the contained yeast is similarly decomposed, so that the still residue contains relatively little nuclein, but a large amount of nuclein bases and free phosphoric acid. This fact must be kept in mind, not only when the distillery residue (from potato and grain distilleries) is used as fodder, but also when the same is used as a yeast food (in the manufacture of pressed yeast) and is added in a certain proportion to the mash, a method which is found by experience to result in an increased yield and greater security in working.

A certain practical value, for the foodstuff chemist, attaches to the reports on the decomposition products of yeast nuclein, in cases where it is a question of determining the purity of a given meat extract and detecting the presence of a substitute prepared from yeast. The quantity of beer yeast annually produced in the great brewing countries has been approximately estimated by Feron at about 305,000 tons. Of this amount about 132,000 tons have hitherto been utilised, partly for pitching in breweries and molasses distilleries, and partly as an adulterant of pressed yeast. This leaves still no less than 173,000 tons unutilised; and many brewers have attempted to find a means of putting this waste product to profitable use instead of letting it run away into the drains or water-courses and thereby leading to constant complaints on the part of neighbours and local authorities. An endeavour has been made to convert this surplus beer yeast, which is rich in proteids and phosphoric acid, into preparations either capable of utilisation as a yeast food in the distillery (especially in molasses distilleries) instead of the usual addition of grain, or else for human consumption as a substitute for meat extracts, or finally as a concentrated cattle food. With this object the yeast is either converted into extract, or is worked up in its entirety alone or with certain adjuncts. A number of processes have been devised for working up yeast into nutritive preparations; and of these, mention may be made of the following for making yeast extracts, viz., those of E. Bauer (II.), Gillhausen, R. Wahl and M. Henius (I.), E. Kressel (I.), Peeters (I.), J. Goodfellow (I.), T. Hill-Jones and E. Kressel (I.), E. Johnson (I.), C. O'Sullivan (I.), D. Watson (I.), A. Denayer (I.), and Lebbin (II.). In the method of G. Eichelbaum (I.), yeast is subjected to the action of species of *Aspergillus* before lixiviation; and according to that of O. G. Overbeck (I.) to the influence of peptase from malt culms. The following methods deal with the yeast as a whole, without extraction: that of Wegener, which is concerned with the production of a coffee substitute from yeast; that of K. Kleinschmidt (I.), and that of Siebel. According to the latter, a pulp which he calls yeast sugar and which has the appearance and consistence of condensed milk, is prepared by grinding pressed and de-bittered beer yeast with sugar and starch. The preparation of concentrated fodder is the object of the methods of J. Steickel (I.) and C. Brucker, as well as of the English Patent No. 20,060 of 1893. The purpose of several of the methods first-named above is the recovery of a yeast extract to serve as a substitute for meat extract; and as a matter of fact some of the preparations of this kind already sold are undistinguishable by the laity from real meat extract, so far as the smell, flavour, and external appearance are concerned. Such are, *e.g.*, Bios, Eurostose and Carnos, according to A. Eichengruen (I.); and, according to Lebbin (II.), Ovos as well. An intelligent manufacturer should obviate all chance of such confusion by giving his yeast preparation a special and indubitable title, *e.g.*, Vegetable Peptone. This, however, is not always the case,

and it would appear that a fraudulent intention is not invariably absent. The foregoing explanations may perhaps divert into a new channel the method of testing adopted by foodstuff chemists for the detection of this yeast extract. By means of the reagents employed in the manufacturing process, and partly also as a result of auto-fermentation, the nucleins of the yeast suffer a more or less extensive degradation, nuclein bases being liberated. Of the latter, adenin and guanin are found in yeast, and consequently occur in comparatively large amount in the yeast extract. On the other hand, as has been shown by A. Kossel (X. and XI.), true meat extract contains little or none of these substances, and is thereby distinguishable from such substitutes. The provision of accurate and reliable data in this respect is reserved for future research in the wide field of the chemistry of the yeast cell.

§ 253.—Glycogen and Fat.

One of the chief phenomena in the metabolism of higher animals is the formation of sugar in the liver. The substance of which this sugar consists was first observed by Claude Bernard (I.) in 1855, and recognised two years later, under the name "matière glycogène," as a carbohydrate similar to vegetable starch (amylum), dissolving in water to furnish a peculiar opalescent liquid, precipitable by strong alcohol, and giving a brownish red stain with iodine solution (iodine 2 grams, potassium iodide 6 grams, and water 120). The occurrence of such a colour reaction had been observed four years earlier by R. L. Tulasne (I.) in the young asci of truffles, and a few years later (II.) on those of *Erysiphe aceris*, De Candolle, a species of the mildew group of fungi. Tulasne seems not to have recollected the observation of his medical colleague, since otherwise he would hardly have contented himself with the briefly expressed hypothesis that a species of albumen with a special affinity for iodine was in question. The next worker in this field, namely, A. de Bary (IV.), in 1863 enlarged our insight into the botanical side of the question by the important discovery that the substance referred to does not appear uniformly distributed throughout the plasma, but is confined to one portion thereof, which he proposed to call the **epiplasma**. At the same time he expressed the opinion that the substance in question was a carbohydrate. Five years later W. Kühne (Ia.) reported the occurrence of the "matière glycogène," or glycogen, in *Aethalium septicum*. This discovery, however, did not afford any certain proof of the appearance of glycogen in fungi, the last-named organism belonging to the *Mycetozoa* (§ 23), and therefore merely a connecting-link between the fungi and the lower animals. Up to that time no definite proof had been adduced of the existence of glycogen in vegetable cells (those of fungi in particular), and therefore the term "animal starch," applied to this substance, was still in general use.

It was not until 1882 that L. Errera (II.), working in A. de Bary's laboratory, showed the principal constituent of fungoid epiplasm to be a carbohydrate, agreeing with animal glycogen in all the properties examined by microchemical means. He also demonstrated its wide distribution, not only in the (first examined) class of *Ascomycetes*, but also in the *Basidiomycetes* and the *Mucoraceæ* (III. and IV.). His discoveries were confirmed by several other workers, *e.g.*, by Krafkoff (I.), and afterwards by E. Laurent (VI.), who found this carbohydrate also in *Oidium lactis*, in the conidia of *Cladosporium herbarum*, and in various rose yeasts.

Of all fungi the yeasts seem to have been the most accurately examined with regard to their content of glycogen, and wherein the metabolic importance of

this carbohydrate has been most clearly recognised. Even for this reason alone, the present appears the most suitable occasion for dealing with this constituent of the fungi. Its microchemical detection presents no difficulties, there being no other known cell constituent that gives a similar deep brown-red coloration in presence of iodine and is therefore capable of being mistaken for glycogen. Even a beginner would not confound the relatively pale yellow furnished, under the same treatment, by the albuminous cell constituents with the full brown-red given by glycogen. Should, however, any uncertainty prevail it may be dissipated by utilising the observation first made by ERRERA (II.), to the effect that if the preparation be carefully warmed to 60° to 70° C. after the addition of the iodine, the pale yellow due to albumen will remain, whilst the red-brown will vanish—to reappear in its original intensity on cooling. If the cells of the treated preparation be ruptured by applying pressure to the cover-glass, a rapid examination under the microscope will show that the browned constituents quickly dissolve in the surrounding fluid on escaping from the cells, the residual portion exhibiting the customary yellow coloration furnished by albuminoids on treatment with iodine. If present in any considerable amount, the glycogen becomes visible to the microscopist by virtue of its optical properties, even in unstained preparations. In such event it is seen in the cells as a semi-fluid, whitish opalescent mass of strong refractive power. Sometimes it takes up a position in such portions of the cell contents as are nearest the walls; but in others, as recorded by ERRERA (V.), it collects in one place and assumes the shape of a semilunar aggregation.

The separation and recovery of **fungoid glycogen** by macrochemical means was first successfully accomplished by ERRERA (II.), though the quantities obtained were insufficient for the requirements of an accurate macrochemical examination. This difficulty was first overcome by M. CREMER (III.) in 1894, who was then able to show that the glycogen of yeast is identical with that in the liver of animals, and that too, not merely in respect of the properties aforesaid, but also with reference to its resistance to the action of Fehling's solution and its behaviour on hydrolysis by dilute hydrochloric acid (as also by diastase, saliva, and the pancreatic enzyme), dextrose being produced. E. SALKOWSKI's report (IV.) that yeast glycogen and liver glycogen differ, inasmuch as the former is partly converted into cellulose on being heated to 130° C. along with a little water, was not confirmed by CREMER (III.). At all events, the optical rotatory power of yeast glycogen was found to be lower ($a_D = +198.9°$) than that of animal glycogen, for which the values in the literature differ and are in part considerably higher (up to 235°).

For comprehensive researches into the chemistry of this carbohydrate we are indebted to a pupil of Errera's, namely, G. CLAUTRIAU (I.), to whose work the interested reader is referred, especially as regards the choice of the best method of isolating glycogen from the different species of fungi. Glycogen is amorphous, and therefore cannot be prepared in the pure state by the crystallisation process, but contains an admixture—larger or smaller in accordance with the origin and care bestowed on the manufacture—of other organic and inorganic cell constituents. This possibility should be kept in mind when mention is made of small discrepancies similar to those found in animal glycogen by earlier workers, and also by Clautriau in comparing yeast glycogen with that obtained from other fungi. Thus the angle of rotation found by this worker, in the case of yeast glycogen, was 184.5°, whereas in that from *Amanita muscaria* it was 196.2°. The same applies to the temperature at which iodine staining disappears; in the case of glycogen from yeast a temperature of 72° to 73° C. is required, whilst in that from other fungi (such as *Boletus edulis*, &c.), decoloration occurs at 58° to 60° C.

There is always the possibility of having to reckon with the existence of several isomeric glycogens, in regard to which see an observation by R. Braun (I.).

The quantitative determination of the glycogen content by the Külz method —which, as modified by E. Pflüger (II.), furnishes reliable results—is a very troublesome task. Clautriau worked out a colorimetric method, the chief features of which consist in dissolving out the glycogen from a weighed (dry) sample, by repeated extraction with alkaline water, then converting the same into its brown iodine compound, and finally estimating the amount by comparing the colour with that obtained by applying the same treatment to a glycogen solution of known strength. In this manner he obtained the following figures, referred to dry residue: 20 per cent. of glycogen in a sample of *Boletus edulis;* 14 per cent. in a sample of *Amanita muscaria,* and 31 per cent. in a sample of yeast. This last figure agrees with that obtained several years earlier in a different manner by E Laurent (VIII.), who found that the richest of several yeast samples contained about 32.6 per cent. of glycogen.

On the instigation of L. Errera (VII.), Laurent also endeavoured to investigate the conditions favouring the enrichment of yeast cells in glycogen, and found that, for this purpose, the method of culture on wort gelatin is particularly useful. The following substances were recognised as glycogen-formers: lactic acid, succinic acid, malic acid, asparagin, glutamine, egg-albumen, peptone, mannite, glucose, lævulose, saccharose, and maltose. According to M. Cremer (I.), these must be supplemented by *d*-galactose and *d*-mannose; but, on the other hand, he found arabinose, rhamnose, sorbose, as well as lactose and glycerine, to be unsuitable; though a contrary opinion with regard to the last two was expressed by E. Laurent. E. Kayser and E. Boullanger (I.) then investigated the influence of other conditions, *e.g.*, the presence of air, and the percentage content of tartaric acid, malic acid, or citric acid, on the inception and progress of glycogen enrichment. In twenty-eight stocks of wine yeast examined with this object, Richard Meissner (III.) found that glycogen makes its appearance, even in the young buds, when the latter have attained about one-fifth the diameter of the parent-cell. A notable observation, and one worthy of further investigation, was made by M. Cremer (VI.), namely, that the glycogen reaction occurred in about twelve hours in a glycogen-free pressed yeast juice that had been treated with 10 per cent. or more of fermentable sugar (dextrose) and kept at the ordinary temperature. Possibly therefore we must assume the presence of a synthetic enzyme in the cell contents.

In view of the amorphous nature of this carbohydrate, some surprise will be manifested at Laurent's report that the yeast cells are capable of absorbing and accumulating the glycogen present in a nutrient solution. And in fact this assumption was contradicted both by M. Cremer (I.) and by A. Koch and H. Hosæus (I.). The last-named observers found in the case of a pressed yeast, a bottom-fermentation beer yeast, and the so-called Frohberg yeast, that the glycogen added to the nutrient solution (wort or meat-extract solution, with or without an addition of grape sugar), whether obtained from calves' liver, rabbits' liver, or pressed yeast, not only remained unutilised, so far as could be ascertained, but also retarded the cell reproduction and fermentative power, so that the yeast crop and the percentage of alcohol in the treated samples were smaller than in those left without addition of glycogen. They failed to detect alcohol in the sugar-free cultures; and concluded that yeast is incapable of secreting a hydrolytic enzyme by means of which the glycogen in the nutrient solution could be converted into fermentable sugar. General application cannot be accorded to this deduction, since M. Cremer (IV.) noted hydrolysis of glycogen on keeping yeast in chloroform water, and found that dextrose was thereby

formed. The contrary assumption, by E. Salkowski (II.), of the formation of lævo-rotatory sugar, is not altogether free from objection, as the latter himself (VII.) admitted. Since the conversion of the accumulated glycogen within the cell is probably connected with such an enzyme, a strong presumption exists in favour of the occurrence of a similar enzyme produced by the plasma. This probability has been brought to almost a certainty by the discovery of Buchner and Rapp (II.) that glycogen can be fermented by expressed yeast juice. The presence of a similar enzyme may also be assumed in other fungi that store up glycogen, and consequently will have to be reckoned with in the separation of this carbohydrate, especially when a quantitative determination of the same is in question. It may be remarked in passing, that, as was first shown by A. Koch and H. Hoseus (I.), the faculty of hydrolysing glycogen is also possessed by various species of bacteria. This at the same time explains Salkowski's (II.) discovery, previously reported by Schützenberger and Destrem (I.), as also the results obtained by N. von Chudiakow (I.), and urged by him against the assumption that autofermentation occurs in yeast.

Glycogen has been rightly termed a reserve material. In times of superfluity of nutrient materials the glycogen is stored up in the cell for the purpose of consumption in case of need, either for maintaining the vitality of the individual in an environment destitute of the necessary foodstuff, or for the construction of new individuals and feeding them until such time as they are able to cater for themselves. This latter event takes place, for instance, when a sclerotium germinates. In the case of *Coprinus niveus* and *Claviceps purpurea*, L. Errera (VI.) accurately traced the gradual migration of the glycogen from the cells of these hard mycelia into the pileated fungi developed therefrom. He also observed the same in the germination of various fungus spores, *e.g.*, those of *Mucoraceæ*. This migration of glycogen is the counterpart of the well-known movement of starch in higher plants, especially during the germination of their seeds. With regard to this worker's report on the formation of glycogen from the oil in the cells, further observations are desirable.

The degradation of glycogen for the generation of energy may either remain only half accomplished, as we shall see in greater detail in a subsequent paragraph dealing with the autofermentation of yeast, or may proceed until the whole is completely consumed into carbon dioxide and water. So far as is known, this condition is reached in the case of yeast cells when the access of air is unrestricted. Cells of wine yeast that have fallen to the ground, keep alive, thanks mainly to their store of glycogen. It does not always happen, however, that the cells are able to use up this store, since they sometimes perish before the same is exhausted. According to the observations of H. Will (X.), dead permanent cells rich in glycogen can always be found in the sedimental yeast of old cultures in wort or (liquefied) wort gelatin. Finally, it may be remarked that the commencement of the working up of accumulated glycogen does not always coincide with the occurrence of a lack of the necessary foodstuffs outside the cell, but seems to be bound up with other circumstances, the age of the cells in particular; so that the glycogen content of a sample of yeast may decline, although sugar is still present in the nutrient solution. In this connection observations have been made by N. Jodlbauer (I.) in the course of some researches which will be referred to in a later section; and these were confirmed by Gontscharuk, whose results have been reported by R. Meissner (III.).

The importance of fats for the life of the yeast cell is equal to that of glycogen; they are also valuable reserve material. Their presence in wine sediment was observed by Braconnot. The percentage of fat varies with the momentary conditions of nutrition of the cells. Payen (II.) fixed it at 2.1 per

cent of the dry residue in the case of beer yeast; but, by the aid of a better method, ensuring complete extraction, Nägeli and Löw (II.) succeeded in separating about 5 per cent. of fat from a bottom-fermentation beer yeast. According to the researches of P. Darexy (I.), alone, and in collaboration with E. Gérard (I.), the chief constituents of yeast fat are stearic acid and palmitic acid (in almost equal amounts), along with a small quantity of butyric acid, part of the acid being in the free state and part combined with glycerine. The chief seat of the fat is in the granules, whose albuminous membrane prevents lixiviating fluids gaining access to the enclosed fat. Consequently, as remarked by Nägeli and Löw, a simple treatment with ether is ineffectual. Still less likely to effect extraction is the small amount of alcohol present in wine; and, in fact, as shown by Müller-Thurgau (XVIII.), wine does not contain more than about 0.1 gram of fat per litre. In a case observed by H. Will (VIII.) in 1898, drops of fat were found in both dead and living cells and spores of a ten-years-old culture of *Saccb. Ludwigii* in beer wort, their colour being reddish yellow, so that the culture gradually became of a brick-red shade.

A substance closely allied to the fats, namely **lecithin**, which is the cholin ester of palmito-stearo-glycerophosphoric acid, and is therefore constituted in accordance with the formula:

$$\underset{\text{Palmitic acid.}}{(C_{15}H_{31}COO)}\ \underset{\text{Stearic acid.}}{(C_{17}H_{35}COO)} : \underset{\text{Glycerine.}}{C_3H_5-O-}\ \underset{\text{Phosphoric acid.}}{PO.OH-}\ \underset{\text{Cholin.}}{C-CH_2.CH_2N(CH_3)_3OH}$$

was first discovered in yeast by Hoppe-Seyler (IV.) in 1866. It was afterwards prepared by him (V.), to the extent of 0.25 gram from 81 grams of air-dry pressed yeast, in the course of rebutting a contrary opinion launched by O. Löw (VIII.) and Nägeli and Löw (II.).

The first discovery of **cholesterin** in fungi was made in 1867, by O. Kohlrausch (I.), in a species of Morel; and soon afterwards it was found by Hoppe-Seyler (IV.) in yeast. Later on, this same worker (V.) recovered 0.44 gram of this monovalent alcohol ($C_{26}H_{13}.OH$) from 81 grams of air-dry pressed yeast. The name cholesterin, which was originally bestowed on a single substance of animal origin, afterwards became enlarged to a collective term, in consequence of the discovery of several isomeric and homologous cholesterins, which, though agreeing with the first cholesterin (from gall, &c.) in their chief properties, yet behaved differently in several reactions. Thus, Hesse isolated from peas the cholesterin to which he gave the name phytosterin; Reinke and Rodewald another (paracholesterin) from *Aethalium septicum;* C. Tanret (IV.) a third (ergosterin), from ergot of rye. According to the researches of E. Gérard (III.–V.), these latter are identical with all the cholesterins hitherto isolated from yeast, as also from *Mucor mucedo*, *Penicillium glaucum*, *Staphylococcus albus*, and indeed from *Cryptogams* generally.

§ 254.—Mucinous Substances of the Carbohydrate Group. The Gelatinous Network.

Under the name yeast gum, several workers have isolated **mucinous carbohydrates** from yeast. Though some of these products have not been closely examined, they have been so far characterised that we may assume the above name to be nothing more than a collective term. They all behave in the same manner towards Fehling's solution, a circumstance of some value in connection

with their preparation and isolation, since all are precipitated by this reagent, as a blue, lumpy copper compound, from neutral or faintly alkaline solutions.

On leaving yeast to undergo autofermentation (*see* a later paragraph) in water, J. Béchamp (VIII.) in 1874 discovered in the liquid (which was protected from invasion by putrefactive organisms) a gummy and optically active substance, the rotatory power of which he ascertained to be + 59 – 61°. This substance first received closer attention at the hands of Nägeli and Löw (II.) in 1878, who prepared the **yeast gum** (or **fungoid mucin** as they called it) by extracting yeast with boiling water, freeing the extract from phosphoric acid and peptone with lead acetate, removing the excess of lead with sulphuretted hydrogen, and precipitating the gum with hot alcohol. Repeated solution in water and reprecipitation with alcohol furnished a white powder, which exhibited a relationship with **dextran** in many respects, *e.g.*, in being precipitable by alkaline copper solution, though its rotatory power was much less, namely + 78°. The formula $3(C_6H_{10}O_5) + 2H_2O$ was deduced from the results of ultimate analysis. This gum was gradually converted into glucose by the action of acids; was stained brown-red by iodine; and therefore—as Errera (II.) surmised with good reason—also contained glycogen.

Wegner (I.) classed as dextran the gum he obtained in 1890 from yeast by the Scheibler method described in § 160. In the dry state this is a white amorphous neutral powder, which is insoluble in alcohol, and swells up in water to an opalescent liquid, the rotatory power of which, referred to about 1 per cent. strength, was found to approximate to $a_D = + 285.7°$.

A **gum** (**lævulan**), differing from the foregoing carbohydrates, was obtained by E. Salkowski (II.) in 1889 by precipitating with Fehling's solution a yeast extract, prepared with water containing chloroform for the purpose of preventing putrefaction.

Whilst the undeniable divergences in the results obtained by the above-mentioned workers compel the assumption that different kinds of gum are present in different samples of yeast, the results of Fritz Hessenland's (I.) experiments permit the further conclusion that several kinds of glutinous carbohydrates may be simultaneously present in one and the same yeast, whether of the top- or bottom-fermentation variety. This worker boiled yeast over a naked flame three times in succession for six hours each, in water containing a small addition of lime, then precipitated with ammonium oxalate the lime from the filtered extract, filtered and concentrated the solution, faintly acidified it with hydrochloric acid, and treated it with an equal volume of 96 per cent. alcohol. The resulting precipitate of brown gum was decolorised by washing with alcohol, and was found to exhibit a percentage composition corresponding to the formula $C_6H_{10}O_5$. The gum from top-fermentation yeast had the rotatory power $a_D = + 283.7°$, that of the product from bottom-fermentation yeast being + 287.6°. The precipitate thrown down from the aqueous solution by Fehling's solution had the composition $2(C_6H_{10}O_5).CuO.H_2O$. This gum furnishes on hydrolysis, not one sugar only, but two, namely, a little glucose and a large quantity of *d*-mannose; it therefore chiefly (but not exclusively) consists of **mannan**, the percentage of which, referred to the dry residue of the yeast, amounts to 6–7 per cent.

E. Salkowski (V.) classed as in the main identical with this mannan the yeast gum which he obtained in 1894 by boiling pressed yeast for half an hour with a tenfold volume of 3 per cent. caustic potash. This treatment brought into solution the whole of the cell contents, including the gum. When cold this extract was syphoned off from the residue (of so-called yeast cellulose), treated with 15 per cent. (vol.) Fehling's reagent, well mixed and heated. The blue

copper compound of the gum separated out in lumps, which were immediately taken out, rinsed with a little water, triturated in a basin with a few drops of hydrochloric acid, and thus converted into a cloudy liquid, from which the gum could be thrown down by a three- to four-fold volume of 96 per cent. alcohol. Repeated solution and reprecipitation finally gave a white, ash-free mass, which was dried with alcohol and ether, and then amounted to about 7 per cent. by weight of the dry residue of the yeast. The results of the ultimate analysis corresponded to the formula $C_{12}H_{22}O_{11}$. In contradistinction to the yeast gum obtained by Nägeli and Löw, this product dissolved readily in water to a filterable, but very glutinous liquid. The sugar furnished by the hydrolysis of this gum seems to have been regarded by Salkowski as *d*-mannose.

Nägeli and Löw thought themselves justified in assuming their yeast gum to be a conversion product of the yeast-cell membrane, because they found that fresh quantities of this gum could be obtained from beer yeast by repeated boilings in water, the total amount (including the so-called cellulose) being about 37 per cent. of the dry yeast. It was then found by E. Salkowski (V.), that the residue left after lixiviating yeast with dilute caustic potash yielded no further gum on prolonged boiling; thus proving that, with a more powerful solvent than mere water, the whole of the yeast gum can be extracted at one operation. This, however, does not solve the problem with regard to the location of this mucinous substance; nor at present is any reliable information available as to the seat of the pentosans, which were detected, to the extent of 2 to 3 per cent. (referred to the dry residue) in both top- and bottom-fermentation yeast, by Hessenland (I.), with the furfural method.

Schützenberger (I.) found in yeast extract a gum, which proved convertible, by hydrolysis, into a reducing sugar. The conclusion that this carbohydrate is an arabin is opposed by the further report that the same was converted, by boiling nitric acid, into a mixture of oxalic and mucic acids. From this behaviour it is probable that he had to do with a galactane. At all events the amount of carbon found, namely, 42.7 per cent., does not correspond with the formula $C_6H_{10}O_5$.

R. Meissner (II.) has described a number of budding fungi, which, when sown in wine must, convert the same into an oleaginous ropy liquid, by the aid of a mucinous substance, the composition and method of formation of which are still unknown. Whereas until very recently the ropiness of beer and wine has been attributed almost exclusively to bacterial activity (§ 164), this worker succeeded in showing that this malady can also be induced by budding fungi. He subjected nine such species to accurate examination, several of them having been isolated by himself from ropy wines and the mucinous exudation from plane-trees, whilst the remainder were isolated by J. Wortmann as pure cultures from the flora of old bottled wines. They are incapable of ascosporulation or of producing alcohol, and therefore do not belong to the family of true *Saccharomycetes*. In a later paragraph mention will be made of the circumstances under which they become a source of danger.

The gummy substances referred to in the foregoing lines make their appearance in quantity more particularly when the fermentative activity of the cells has drawn to a close. In this event, whether as a result of the swelling of the invariably mucinous outer layer of the membrane, or of an excretion from the interior, the mucinous envelopes developed by the individual cells coalesce to a sort of honeycomb in which the cells appear embedded. These formations were first observed by E. Ch. Hansen (X.), who gave them the name "gelatinous network." They were found in the so-called yeast ring and in the films (§ 246) on old cultures of *Saccharomycetes* and a few other budding fungi, grown in

nutrient solutions. They are also not infrequent in the inoculation streaks that have been drawn on gypsum blocks for the purposes of spore analysis (§ 247); and a similar gelatinous network is often observable in yeast samples (about the size of a pea) that have been taken by the brewer and dried between blotting-

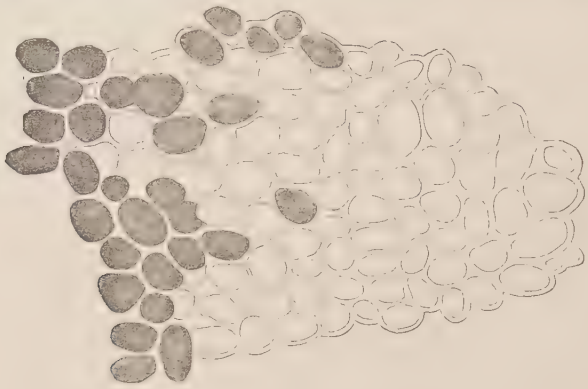


FIG. 157.—Network in Carlsberg Bottom-fermentation Yeast No. 1. Hansen.

The majority of the cells have been washed out of the network in the course of staining with Methyl Violet, only thirty-one cells being left. These, having been deeply stained, have a dense black appearance in the Fig. Magn. 1000. (*After Hansen's original drawing.*)

paper in order to be conveniently sent by post to a laboratory for examination. Their detection is facilitated by the aid of staining, either with Methyl Violet, which stains the cells only (Fig. 157), or by the so called capsule staining method (§ 33) described by HANSEN (XVI.). The latter method stains only the mucinous network, and thus renders the latter visible in cases where it could not

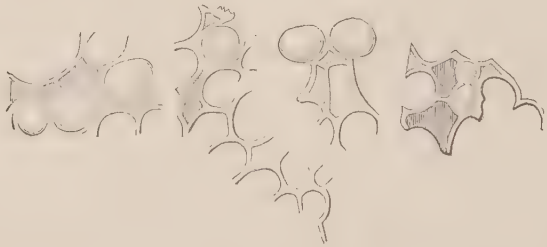


FIG. 158.—Fragments of Gelatinous Network.

From a beer yeast. Unstained. Magn. 1000. (*After Hansen's original drawing.*)

be detected in the unstained preparation. It sometimes happens that, during the preliminary treatment of the sample of dried cells to be stained, the cells themselves get washed out of the network, thus leaving the latter isolated and empty. Fragments of evacuated network are not infrequently observed (Fig. 158). Nevertheless, it should not be assumed, without further inquiry, that every formation with this appearance is a residue of this kind; but one must bear in mind the observation made by H. WILL (VII.) to the effect that the glutin bodies present in sedimental yeast frequently exhibit, when dried, an appearance resembling fragments of the network in question. In such cases, decisive information is afforded by an addition of acetic acid to the preparation,

this acid dissolving only the remains of the glutin bodies. Will has also shown that several different kinds of reticulation, and substances composing same, are found in the films and yeast ring of wort cultures of beer yeast. This matter will be reverted to it in the next paragraph.

The addition of a large quantity of water to the yeast dissolves the network, Nevertheless, as Hansen has shown, it forms anew, unless the treatment be repeated sufficiently often and prolonged. According to H. Will (VII.), however, it is permanently removed when the washing is repeated ten times in twenty-four hours. Now, since the percentage of the constituents forming the network in beer yeast (and also pressed yeast) influences the compressibility of the mass, it follows that this property may under certain circumstances be impaired by the method pursued for washing the yeast (§ 255). On the other hand, it becomes indispensable to remove these mucinous constituents from the yeast when the latter has to be subjected to a quantitative macrochemical analysis for the purpose of ascertaining the composition of the corpus of the cell. This purification might be effected by the washing process, were it not that the treatment so far modifies the cells themselves as to make the results of the analysis, under certain circumstances, not worth the trouble expended thereon.

In this case also, practical use may be made of the general property of the vegetable gums for thickening to a jelly in presence of borax solution, and thus separating from the liquid. On the basis of an observation by Dumas (III.), H. Will (I.) found that a thick barm of beer yeast, when treated with a 5 per cent. solution of borax, immediately agglomerates into pure white lumps, which quickly subside and unite into large solid masses that fairly crackle when spread out on plates of gypsum. This behaviour is advantageously utilised in the production of pure-culture yeast on a practical scale. The thick barm of yeast from the fermenting cylinder of the pure yeast apparatus is treated with a similar solution of borax, when destined to be sent out in a pressed state. This treatment not only accelerates the deposition of the yeast and considerably facilitates pressing, but also greatly ameliorates the colour of the yeast, and therefore improves its appearance, without impairing the fermentative power to any appreciable extent. It would therefore seem that the precipitated mucinous matter carries down the yeast cells, but not the brown hop resins, &c.

The foregoing explanations may also prove useful in practical analytical chemistry, namely, in cases where the percentage of sugar in wine and similar liquids is to be determined with the aid of Fehling's solution. In such event it should be remembered that this reagent also precipitates the gummy substances present, the resulting precipitate of cuprous oxide also including the precipitated copper compound of the gum; consequently too large an amount of copper is weighed, and the quantity of sugar calculated therefrom will be far in excess of the truth. This applies more especially to the so-called yeast wine, which is an inferior wine obtained by pressing the sediment (the so-called wine yeast) from the wine casks. This sediment is very rich in gum, and therefore (as was shown by K. Amthor (III.)), should not be examined for its sugar content, by the aid of Fehling's solution, without bearing this in mind. With the said yeast wine, in this respect, must be classed the fruit wines (mostly very rich in mucinous matter) and such grape wines as have been racked from the yeast very late, and have therefore absorbed large quantities of gum from the latter during the protracted period of contact therewith. It may also be mentioned that certain gummy substances find their way from the grapes into the wine must, and that experiments have been made by G. Nivière and A. Hubert (I.) with regard to the gum recoverable from wine. Gummy substances are also found in malt,

having been isolated therefrom by C. J. Lintner (IV.). It may occasionally happen that such a gum, when not eliminated during mashing and boiling, renders the beer dichroic, instances of which kind have been reported by H. Will (XI.).

§ 255.—Albuminoid Mucinous Substances. "Head" and Frothy Fermentation.

The network enveloping the yeast cells is not invariably composed of the carbohydrate gums described in the preceding paragraphs. Careful investigations performed by H. Will (VII.) have shown that, in the case of a considerable number of stocks of beer yeast, the enveloping, agglomerating network is constructed of a material that gives all the reactions of albumen. This albuminous matter is more abundant in the upper layer of the sedimental yeast found in the fermenting tun at the close of primary fermentation, but is less plentiful in the lower or core yeast; and it differs from the other albuminous admixtures present therein, not only in its lack of definite form, but also in point of origin. From the fact that it is also encountered in cultures grown in nutrient solutions free from albumen, one may conclude that it is elaborated by the yeast cells themselves. Of course the further question must be left undecided whether, in nutrient solutions that already contain albumen, the amount is increased by some portions of this latter substance.

Beer yeast (sedimental yeast) when well shaken up with ether (1 : 1), takes up a certain quantity of the same, the amount depending on the percentage of this albumen and the aforesaid mucinous substances present. Yeast from the upper layer takes up two to three parts by volume of ether, but core yeast only about one part, the mucinous substances forming bubbles charged with ether. In these laboratory experiments the ether plays the same part as carbon dioxide does in practical fermentation in the vat; and as the mucinous substances do not remain in the sedimental yeast, but are in part ceded to the supernatant liquid, they are able, in the above-described manner, to effect a firmer retention of the carbon dioxide than can be done by mere physical solution. Consequently, under certain circumstances, they may considerably augment the so-called permanent absorptive capacity for carbon dioxide on the part of the beer.

The fact that a soluble albuminoid takes part in the formation of the "head" or scum thrown up by fermenting wort was first suspected by Habich, and then proved, by isolation, by C. Lintner, sen., and Reischauer in 1876. This **froth glutin (Krauesenglutin)** is said to originate in the wort or the malt. By means of comparative fermentation experiments with different beer yeasts, it was then established by Alb. Reichard (III.) that, in addition to this malt albuminoid, the presence of certain mucinous excretions (especially those of an albuminoid character) from the yeast is essential to the formation of a normal "head" composed of fine bubbles. The condition of these formative materials in the head at different stages of fermentation has not yet been more closely investigated.

In the case of a liquid absolutely devoid of viscosity and free from mucinous constituents, the bubbles of carbon dioxide liberated in the interior would immediately unite to large ones and make their escape without delay as soon as they arrived at the surface. By means of these mucinous substances, however, each bubble of gas is surrounded by a tough envelope at the instant of liberation, and thereby prevented from becoming merged into others. These bubbles collect on the surface of the liquid, stick together, and thus form by degrees the fine

permanent "head" so desired by the brewer and the pressed-yeast maker. The ascending bubbles also carry to the surface other constituents of the fermenting wort, and incorporate them with the structure of the head, chief among these being yeast cells (*see* below), then fragments of tissue from the mashed materials, and finally (in the case of hopped wort), glutin substances and globules of hop resin, to which latter the disagreeable bitter flavour of the head is due. A permanent head of the kind is peculiar to beer, and is not produced in the fermentation of unhopped wort or on wine must; and one must therefore conclude that the presence of hop constituents is indispensable to the structure of a normal head on beer. The accuracy of this conclusion has been rendered more probable by the results furnished by the experiments of E. Ehrich (I.).

The thickness of the head thrown up by the activity of yeast in wort, mash and wine must, is dependent—other conditions being equal—on the available supply of the materials, by means of which the initially naked gas bubbles are converted into mucinous bubbles charged with gas. However, the amount of these substances produced by different stocks of yeast stands in no definite relation to their fermentative activity, *i.e.*, the amount of carbon dioxide liberated in unit time. Hence it will occasionally happen, in comparative fermentation experiments, that a relatively high loss in weight (*i.e.*, liberation of carbon dioxide) is noticed in specimens which, from their appearance during fermentation, would seem to have fallen behind considerably. Thus, for instance, in a set of experiments conducted by Müller-Thurgau (IV.) with 25 stocks of fruit and wine yeasts, the one (a Wädensweil cider yeast) that liberated the largest amount of gas fermented its nutrient substratum without the slightest formation of head. So far, no close attention has been devoted to the capacity of the wine yeasts for depositing albuminoid mucinous substances, though, in addition to the above-named, other observations have been recorded indicating the existence of this capacity. Thus, for example, the well-known loss of colour in red wines when treated with white-wine yeasts seems attributable to the action of similar albuminoids. In contrast to the red-wine yeasts, which are already saturated with colouring-matter, the albuminoid mucinous constituents of white-wine yeasts are still in a position to take up tinctorial substances. The case is analogous to that of browned wines when treated with yeast, this procedure, according to Nessler (III.), affording a remedy for the malady in question. The combining and precipitating power of these albuminoid excretions of yeast is probably the cause of the phenomenon—well known in practice, and recommended for utilisation by H. Müller-Thurgau (III.)—that a wine rendered turbid by bacteria can be cured by pitching with a small quantity of must in active fermentation. This must is very rich in yeast cells, Müller-Thurgau (V.) having detected about 3 milliards per litre. P. Lindner (XVI.) has reported a similar instance of the cure of a sarcina-ropy white beer by adding yeast and rousing.

The chief practical difference between top fermentation and bottom fermentation is due to the aforesaid relation between the yeast cells and the mucinous substances, either produced in the nutrient medium or excreted by the cells themselves. A typical example of top-fermentation yeast in its fullest development is afforded by the stocks forming the chief component of unadulterated pressed yeast. The main features in the preparation of this article by the old or Viennese method are as follow: About three to four hours after the wort has been pitched with yeast—a so-called "artificial" yeast (§ 148) of suitable quality—the mash begins to work. The scum of husks and grains that has accumulated on the surface in the meantime is now penetrated by an ascending white head, the development and growth of which, during the next twelve hours,

presents a picture of ebullient motion of considerable briskness. Under ordinary circumstances the head at the end of this time will have attained a thickness of twelve to fifteen inches, whilst the gravity of the wort will have decreased to about half its initial value. This head forms the vehicle containing the major part of the yeast crop reproduced from the pitching yeast. The bubbles are still transparent. Whereas, during the next three to four hours, the head does not increase in thickness to any great extent, the young cells therein begin to grow; this stage is termed the ripening of the head. The bubbles begin to turn cloudy, and finally reveal the presence of large yeast colonies which, to the unassisted eye, appear as white spots. The reproduction of the cells is now ended; the vat is ripe, to use a practical term; and the gravity of the wort has fallen to about one-third the original strength. The head is next skimmed off by means of suitable skimmers, and is transferred to a sifter in order to separate the coarser adherent particles of the grains, the elimination of the finer undesirable admixtures being effected by the subsequent washing, to which reference will be made in the paragraphs dealing with the autodigestion of yeast. For the present we will merely utter a warning against a one-sided judgment and imperfect appreciation of the importance of the mucinous excretions in connection with facilitating the ascent of the yeast in the fermentation vats during the manufacture of pressed yeast, by calling attention to the influence exercised by the viscosity and chemical constitution of the mash on the working of the yeast, and therefore on the yeast crop. More detailed information on this matter will be found in the volume on pressed-yeast manufacture, issued by O. Durst (I.).

The so-called riotous or bladdery fermentation of beer yeast may be left out of consideration here. The structural materials for the large, tough bladders forming the head in this case are not supplied by the yeast, but from the sludge—as was shown by the researches of A. Reichard (III.). One of the physiological causes determining the appearance of this undesirable phenomenon will be found mentioned in § 258.

Furthermore, only a few words can here be devoted to the so-called frothy fermentation which occurs in spirit distilleries, because the causes of this phenomenon have not yet been sufficiently investigated to furnish results that could be dealt with in the limits of the present work, or permit any reliable conclusions to be drawn. Nevertheless, all the observations on this point recorded in practice may be mentioned as affording noteworthy hints for the more accurate examination of the matter by fermentation physiologists. Special attention may be drawn to the observations recorded by M. Delbrück (I.). In the normal course of fermentation in potato distilleries, the upper layers of head formed on the surface of the mash disappear in proportion as fresh portions are thrown up from below; whereas, when frothy fermentation occurs, the upper bubbles do not burst, but the thickness of the head goes on increasing, until the entire contents of the vat seem to be in a state of ebullition, and thick masses of froth overflow the top of the vessel. This is a direct source of loss, the more so in countries where the excise regulations prohibit the recovery of the overflow. In many cases the evil may be either entirely remedied, or reduced to a minimum, by pouring a little oil into the contents of the vat as soon as frothy fermentation becomes manifest. Yeasts of high fermentative power are especially liable to this evil, *e.g.*, the so-called Race II. already mentioned (vol. ii. p. 81); though in other respects this is a very useful yeast. A few observations on this point have been communicated by M. Bücheler (I.), and further information thereon is contained in M. Märcker's handbook on the manufacture of spirits (I.). Undoubtedly, the occurrence of frothy fermentation is determined by special physiological conditions, based on the method of nutrition of the

yeast: for example—according to R. Kusserow (I.)—the simultaneous presence of large amounts of amides and peptones in the nutrient substratum. This has been borne in mind in arranging a modification of the mashing process in cases where there is a possibility of this frothy fermentation being set up. According to the method proposed by Hesse, a distillery manager, of Wutzig—as reported by M. Delbrück (II.)—only one-half of the malt requisite for the saccharification process is introduced into the warm mash, the remainder being added after cooling and the introduction of the pitching yeast. The results of this treatment are reported by G. Heinzelmann (I.) to be satisfactory, almost without exception. In pressed yeast works it also occasionally happens that riotous fermentation is set up in the vats. With regard to the incorrectly named frothy fermentation occasionally met with in the *masse cuite* and after-products of sugar works without any recognisable cause, and which is regarded by some technicists as a purely chemical process of decomposition, some information will be found in a communication by O. Laxa (I.).

The fact, of which mention has already been made, that certain albuminoid constituents are extracted from yeast cells by ethyl ether, has been utilised in practice by H. Buchner and M. Gruber (I.), who manufacture nutrient preparations therefrom by a patented process.

§ 256.—"Break" and Clarification in Beer and Wine.

In the formation of head on fermenting liquids the aforesaid mucinous excretions from the yeast cell are associated to a greater or smaller extent, according to circumstances, with other mucinous bodies already initially present in the nutrient substratum. Their dependence on such assistance is diminished by the inception of the phenomenon known as "break" in brewing and wine-making.

In proportion as the liberation of carbon dioxide gradually decreases towards the end of fermentation, there ensues a diminution of the force by means of which the yeast cells floating in the fermenting liquid have so far been kept in motion. Consequently the attraction of gravitation can now make its influence on the cells, which have a higher specific gravity than the young beer or new wine. The resulting subsidence of the cells is, however, opposed by two forces, namely, the viscosity of the liquid and the friction between the liquid and the individual cells. These forces suffer diminution when several cells adhere to form a small ball, since, whilst the weight of this agglomeration is equal to the sum of the weights of the individual component cells, the surface is considerably smaller than the total superficial area of the component parts. Hence, while the downward pressure of the weight remains the same as before, the surface of contact with the liquid, and therefore the amount of resistance, is less. Now the aforesaid gummy and albuminous mucinous matters favour, and indeed are indispensable to, this agglomeration of the cells. When they have accomplished this task and united the cells into colonies that are visible to the unassisted eye, the condition known to the brewer as "break" is attained. This condition, which gradually arises towards the end of primary fermentation, forms, in conjunction with the gravity of the wort, the most important factor for determining the ripeness of young beer, and on this account is carefully observed by the brewer. When the process has gone so far that the initially cloudy wort shows up clear in the sampling-glass, with the yeast collected together into small, gravelly lumps, the brewer is in a position to order the beer to be racked into the storage cask, and thus separated from the sedimental yeast. According as the racking is effected at an earlier or a later period, so a larger

or smaller number of yeast cells will be carried over into the storage cask; and the beer is said to be racked green or bright respectively. The latter condition is the more desirable, the exact gradation, however, varying according to circumstances.

The progress of clarification in lager-beer, that is to say, its self-purification from the yeast cells, is shown in the following figures, which were determined in 1896 by F. Schönfeld (I.) in Berlin lager-beer, by means of the yeast counter (§ 83).

One Cubic Millimetre of	Yeast Cells
Freshly pitched wort contained	14,600
Young beer previous to racking contained	54,000
Young beer, racked somewhat green „	2,000
Young beer, racked bright „	500
Lager-beer ready for drinking „	0.1 to 10

Thus, at the end of the primary fermentation, the number of yeast cells carried over into the storage cask amounts to 5 per cent. when the "break" is fairly good, and to 1 per cent. when the same is very good. Similar results were obtained for North American lager-beer in 1889 by R. Wahl (I.). In order to facilitate the deposition of these yeast cells in the storage cask, it is the custom to place in the cask a number of shavings or strips, either of hazel wood or sheet aluminium. This has been reported on by H. Will (XVIII.). On this foundation the cells adhere firmly, and the microscopical examination of such strips from the empty storage cask shows that the cells are, as it were, glued on to the strips by the mucinous substances already referred to. Imperfectly clarified beer is said to suffer from yeast turbidity or haze, and should not be offered for sale, since, even when the yeast is in small amount, it is often the cause of gastric catarrh, diarrhœa, and indeed slight toxic symptoms, in the consumer. The actual and proximate causes of this action are still undetermined. The literature of this matter will be found collected in a useful work by N. P. Simanowsky (I.) and in a communication by R. Schwanhaüser (I.), a few experiments on the same point having also been undertaken by J. Neumayer (I.). With regard to the clarification of cloudy beer by filtration, reference may be made to § 75 in vol. i. The proposal made by Otto Reinke (I.) in 1896, to facilitate the clarification of beer by the aid of light, can hardly be recommended, since, as already shown by W. Schultze (I.) in 1888, the agreeable flavour of the beer rapidly disappears under such treatment.

The commencement and development of "break" form the chief criterion whereby the small brewer is able to pass judgment on new stocks of yeast, purchased from outside sources and in use for the first time. This circumstance, it may be remarked in passing, forms the chief reason for the difficulty experienced in introducing pure-culture yeast into the smaller breweries, it being a peculiar feature of many stocks of pure-culture bottom-fermentation yeast that, on their first introduction into the brewery, they exhibit little or no "break" until they have been pitched on two or even three successive batches of wort. Now, it is easy to understand that, in presence of this difficulty, the brewer, being already suspicious of the value of the innovation, should decide to revert to the "good old stock yeast" to which he is accustomed, oblivious for the moment of the many disappointments the latter has already caused him. The causes of imperfect "break" are manifold, and, in part, still undetermined; for instance, an insufficiency of lime (§ 258) in the wort, too low a fermenting temperature, or imperfect rousing during the cultivation in wort. On the other hand, some stocks of yeast exhibit a defective "break" under all conditions, and furnish a thin sedimental yeast instead of a firm deposit in the tun. Despite

the greater care involved in working with yeasts of this type, they are still employed, especially when they exhibit other valuable qualities and furnish, for example, a beer characterised by great stability and therefore suitable for export. Such a yeast is met with in the Carlsberg bottom yeast No. 1, by means of which E. Ch. Hansen first introduced his pure-culture method into the fermentation industry. On the other hand, for the production of quick-running ales, it will be found economically advantageous to make use of a yeast that clarifies rapidly.

Yeasts exhibiting coarse "break" and good clarification are still more important in the preparation of champagne than for brewing, since in the former case imperfect clarification cannot be remedied by the use of strips or by filtration. Presupposing a knowledge of the rudiments of champagne-making, it may be recalled that the wine for this purpose is treated with sugar, and is subjected whilst in bottle to a secondary fermentation in order to produce the requisite amount of carbon dioxide. In the old process the fermentation of this sugar was left to the few cells usually present in the wine; the results, however, frequently failed to come up to the expectations formed in this respect. The defect can be remedied by adding a sufficient quantity of a pure-culture yeast to the sugared wine (cuvée), which yeast must of course be specially selected for the end in view. Thus, it must not only possess a high power of resistance towards the presence of a large amount of alcohol in the nutrient substratum, and to a high gas pressure, but also have the property of settling down in granular aggregations when fermentation is completed, so that the whole of the yeast separates completely when the bottle is degorged. Now, many of the yeasts present in the wine itself are incapable of fulfilling this last requirement, and for this reason waste has hitherto been inevitable. The yeast refuses to "shake on to the cork" in a satisfactory manner, *i.e.*, it cannot be induced to rest in position above the cork by the usual process of carefully tapping and turning the inverted bottles, but adheres in patches on the walls and cannot be dislodged: thus making the champagne of inferior value, or even quite unsaleable. However, thanks to the endeavours of H. Müller-Thurgau (IV. and V.) and J. Wortmann (V.), champagne manufacturers are now able to obtain pure yeasts which will shake on to the cork properly, and collect as a gravelly deposit without adhering to the sides of the bottle. The removal of the yeast is therefore attended with far less waste of liquor than was formerly the case; and the same may also be reduced to a minimum by means of the process patented in Germany (No. 60,351) by Walfard. In this method the granular deposit of yeast is congealed by immersing the corked bottles in a refrigerating mixture at a temperature of about -20° C.; on the bottle being then set upright, the yeast and cork (which has contracted under the influence of the cold) are easily expelled together by the immediately augmented pressure of the carbon dioxide.

The highest demands on the coherence of the yeast colonies are made on the stocks used for the purposes of the sparkling fermentation introduced into practice by H. Müller-Thurgau (V.). In order to increase the stimulative effect on the consumer's palate, it has become more and more the custom of late years to make thinner grape and fruit wines, poorer in flavouring extractive substances, but rich in carbon dioxide or "sparkling." This enables one to dispense with the artificial enrichment with carbon dioxide (carbonation) already extensively practised (according to Nessler (III.)) in the Moselle district, sparkling fermentation being a more efficient substitute therefor. The clear or clarified wine, which has either been poor in gas from the outset or has become flat through repeated rackings, is treated with an addition of 300 to

500 grams of saccharose per hectolitre, and the whole is then pitched with a pure yeast that remains at the bottom throughout the entire period of gas formation, which soon begins. Contrary to what happens during fermentation in champagne bottles, the fermenting liquid retains its brilliance undiminished all the time, and when finally matured and sparkling can be readily and effectually separated from the settled ferments. Reihlen, of Stuttgart, many years previously arrived at the same result by different means, namely, by the use of what he termed "fettered" yeast, prepared by impregnating fibrous material (*e.g.*, wood pulp) with must and sowing it with yeast. When development was complete, he washed off the superficial shells and introduced the "fermentation fibre" (richly interspersed internally with fettered cells of yeast) into the wine cask, where it carried out the desired secondary fermentation without affecting the brightness of the liquid.

SECTION XIII.

YEAST NUTRITION AND YEAST CULTURE.

CHAPTER XLIX.

MINERAL FOODSTUFFS.

§ 257.—Ash Content and Ash Analysis.

Since the requirements of the Eumycetes in general in respect of ash constituents have already been fully dealt with in chapter xli., it may seem superfluous to the reader to revert to the question of the mineral needs of the yeasts, all of which belong to that group. It should, however, be remembered that the matter was then treated from a purely physiological standpoint, the problem being to ascertain what mineral substances are essential to the structure of the Eumycetes, and therefore also yeasts. The question, however, comes into the region of practical economics, so soon as we have to deal with ferments (*e.g.*, yeasts) that are required in large quantities for industrial purposes. In this case it is no longer sufficient to know that certain ash constituents are indispensable, but it also becomes necessary to ascertain how these requirements can be satisfied in practice, and to investigate the conditions under which the activity of these ferments can be raised to the maximum by a suitable selection of the sources of supply of the mineral foodstuffs under consideration.

From this practical standpoint we shall deal more fully, in the present chapter, with three elements: calcium, phosphorus, and sulphur, which have been, up to the present, more fully investigated than any others with regard to their influence on the development and activity of yeast. On the other hand, our knowledge on the importance of potassium must be characterised as scanty, and therefore this foodstuff (also known to be essential) must necessarily be dismissed in a few words. From experiments conducted by H. Becker (I.) it appears that the quantity of potash in the nutrient solution influences the degree of attenuation. A beer wort containing naturally 0.071 per cent. of potassium (K), attenuated to 56.4 per cent., whereas in the case of two parallel samples, the potassium content of which was raised to 0.078 and 0.085 per cent. by the addition of potassium carbonate, attenuated, under similar conditions, to only 52.2 and 48.9 per cent. respectively. Hence the increased potassium content of the wort resulted in a reduced attenuation. In tests carried out by R. Kusserow (I.), the addition of dipotassium phosphate (K_2HPO_4) had no apparent influence on the fermentation of the wort; but here the effect of the potassium alone could not be judged, owing to the simultaneous presence of the phosphoric acid of the salt. According to the observations of C. G. Matthews (II.), potassium is more readily assimilated when a portion is present in the condition of sulphate than when the phosphate alone is used. Compare also the statement of A. Mayer (I.) on this point.

Before proceeding to discuss the importance of the aforesaid three elements in connection with the life and action of yeast, it will be necessary to deal with

two points of special interest to the chemist and fermentation technologist, namely, the percentage and the quantitative and qualitative composition of the ash constituents of yeast.

With regard to the ash content, numerous determinations are available, a few of which, referred to the dry matter of the yeast, are given below:

PERCENTAGE OF ASH IN YEAST.

Top fermentation Yeast . . .	7.65	2.5	8.9	5.5	11.5
Analyst—	Mitscherlich.	Schlossberger.	Bull.	Bèlohoubek.	HESSENLAND (I.).
Bottom Yeast	7.51	3.5	5.3	8.1	8.07
Analyst—	Mitscherlich.	Schlossberger.	Wagner.	Schützenberger and Destrem.	C. Lintner, Munich
Bottom Yeast (*cont.*). . . .	7.61	8.76	7.7	10.1	
Analyst—	C. Lintner, Weihenstephan.	Seyffert, St. Petersburg.	BÉCHAMP (VI.)	Hessenland.	

These figures are not all equally reliable. For instance, the remarkably low figures given by Liebig's pupil, J. SCHLOSSBERGER (I.), are due to the fact that the yeast samples were not only washed very clean with plenty of water, before drying and incinerating, but were also "purified" with cold and hot water, and with ether, as though dealing with a precipitate of the character of ferric hydroxide, instead of delicate cells with permeable walls. Other workers, though proceeding with greater care, have mostly overlooked the fact that the sedimental yeast they obtained from the brewery for the purposes of their experiments does not consist exclusively of yeast cells, but contains all sorts of other matters (§§ 245, 254, 255), only a few of which are removed by washing and sifting, so that a considerable difference exists between the ash of yeast, *per se*, and that of ordinary sedimental yeast.

The foregoing objections apply equally to the percentage composition of the two kinds of ash; and for this reason the percentage results of the series of ash analyses performed on wine yeast by Braconnot must be omitted. Furthermore, the results of analyses of commercial pressed yeast, by CHAMPION and PELLET, and A. BÈLOHOUBEK (II.), in the table (vol. ii. p. 141) must not be taken as unconditionally accurate, since this class of yeast contains a considerable amount of (ash-bearing) starch, the proportions being in these two instances 10 and 14 per cent. respectively.

For the purpose of criticism we will take the first column of figures. The 53.8 parts of phosphoric acid require 107 parts of lime for complete saturation, whereas only about 40 parts of that substance are present, *i.e.*, only sufficient to neutralise 21.6 parts of P_2O_5. The remaining 28.4 parts cannot be completely combined by the other bases, viz., 1.0 CaO and 6.0 MgO, so that $21.6 - (0.8 + 7.1) = 13.7$ parts of P_2O_5 are left in a free state. This excess of free phosphoric acid accounts for the acid reaction of yeast ash, or the solution thereof, which reaction was first observed by QUEVENNE (I.). Being able to resist the action of heat, this free phosphoric acid effects the expulsion of sulphuric acid during incineration, and consequently the latter acid will not be found in the ash unless measures have been taken to ensure its combination and protection. Such measures were first adopted by BÉCHAMP (VI.) in 1871, but were neglected by MITSCHERLICH (III.) in 1845, and even by J. LIEBIG (II.) in 1870, although it was already known that yeast contains an appreciable quantity of sulphur. In fact Liebig himself found 0.69 per cent. of sulphur in the dry residue, whilst Mitscherlich, Reichenbach, and Dempwolff gave the figures 0.6, 0.57, and 0.39 per cent. respectively. The same objection must be made with regard to the results of analyses by H. Seyffert, given in subsequent paragraphs.

PERCENTAGE COMPOSITION OF YEAST ASH.

	Pressed Yeast.			Top Yeast from White Beer.	Bottom Yeast, Munich.			Weihenstephan Bottom Yeast.			
	Mitscherlich.	Bèlohoubek.	Champion and Pellet.	Bull.	Liebig.		Mitscherlich.	Béchamp (VI.)		Lintner.	
K_2O	39.5	38.68	23.33	35.2	29.1	30.6	28.3	28.79	31.52	38.45	26.07
Na_2O	—	1.82	16.63	0.5	2.5	—	—	1.93	0.77	—	2.26
CaO	1.0	1.99	1.36	4.5	2.4	2.1	4.3	2.49	2.39	2.85	7.58
MgO	6.1	4.16	5.23	4.1	4.1	4.2	8.1	6.54	3.77	5.80	6.34
Fe_2O_3	—	0.06*	*	0.6	—	—	—	7.34?	2.73**	0.51	0.70
P_2O_5	53.8	51.09	49.06	54.7	44.8	48.5	59.4	53.87	53.44	48.19	54.31
SO_3	—	0.57	trace	—	2.1†	—	—	6.38	5.05	0.62	0.31
SiO_2	trace	1.60	1.88	—	14.4	—	—	trace	trace	1.26	0.92
Cl	—	0.03	trace	0.1	—	—	—	?	?	—	—
Not determined	—	—	††2.15	—	—	—	—	—	—	—	—
	100.4	100.0	—	99.7	99.4	85.4	100.1	107.34	99.67	97.68	98.49

? An error. * Trace of MnO. † Incl. Chlorine and CO_2. ** Trace of Al_2O_3. †† Incl. Fe_2O_3.

In connection with the phosphoric acid, careful procedure is also necessary to prevent its partial reduction to (volatile) phosphorus during the initial carbonising stage of the incineration process. The possibility of this loss is apparently not precluded even in the Mitscherlich method. With regard to the 14.4 per cent. of silica mentioned in the fourth column, it is uncertain whether this is entirely due to accidental contamination of the sample with sand, &c. (vol. ii. p. 36). It is therefore evident that it is by no means an easy task to secure a perfect analysis of yeast ash; and it is desirable that the hiatus, resulting from this cause, in our knowledge of yeast and its vital activity, should be filled up as early as possible.

§ 258.—The Importance of Lime.

We cannot expect to find any reliable particulars regarding the ash requirements of yeast at a period when the authorities were of opinion that this "ferment" was nothing more than a proteid body in a state of incipient decomposition. Even T. A. Quevenne (I.), who did not dispute that yeast possesses the characteristics of a living organism, regarded the ash of same as something quite immaterial—an accidental impurity.

Since, as already mentioned, the proteids also contain ash, it was not possible to affirm the necessity of mineral foodstuffs for the development of yeast, until a nutrient medium had been discovered in which the nitrogenous food constituents were present in a state of combination free from ash. This was first successfully accomplished by Pasteur (VII.), who proposed to employ the readily procurable ash from beer yeast for the purposes of artificial culture in nutrient solutions in the laboratory.

The further problem of the number and character of the indispensable inorganic elements was not attacked by Pasteur, the first to do this being Adolf Meyer (I. and V.), who ascertained, by numerous culture experiments, that the mineral foodstuffs indispensable and sufficient for the development of yeast are potassium, magnesium, iron, phosphorus and sulphur.

The resulting indirect assumption that lime is unessential conflicts with the practical experience, of brewers especially, that worts and mashes poor in lime give a very defective fermentation. In view of this circumstance, the preceding sentence requires modification in so far that, though lime may not be essential to the actual growth of yeast, it forms an indispensable adjunct or stimulant when the fermentative power of the yeast is concerned. The part played by lime in this connection is still unknown, and its investigation would be a very thankworthy task. Possibly it combines with and nullifies the action of the poisonous oxalic acid, which was shown by C. Wehmer (V.) to be a common and important final product of the metabolism of numerous fungi, including *Aspergillus glaucus*, *A. niger*, *Penicillium glaucum*, *Mucor mucedo*, *Rhizopus nigricans*, *Phycomyces nitens*, *Peziza Fuckeliana*, &c. In the case of these fungi, the acid can probably be rendered innocuous by the magnesium present, though the larger quantities produced by the far greater fermentative activity and substantive transformations resulting in the case of yeast, cannot be properly dealt with by that base alone. A more careful examination (which does not seem to have been undertaken as yet) of the gradual increase in formation of oxalic acid during the fermentation of beer wort, and the determination of the nature of the base, or bases, with which this acid is combined, will, it is hoped, bring us a step nearer to solving the problem of the importance of lime in connection with yeast.

As the result of wide experience, brewers are agreed that yeast which has been grown in worts poor in lime quickly degenerates, and in particular is

incapable of producing "break" in the wort. This defect is frequently experienced in breweries compelled to use very soft brewing liquor (*i.e.*, deficient in lime), and it is a common practice to counteract this evil by adding a little powdered (unburned!) gypsum to the mashing liquor (about 1½ oz. per 1000 galls.). Very satisfactory results are obtained: good head, thorough fermentation, good "break" and a firm sedimental yeast (provided the yeast employed is capable of furnishing such, *see* vol. i.). We are indebted to H. SEYFFERT (I.) for an interesting example, relating to a Saint Petersburg brewery, working with a liquor containing only 1.3 parts of CaO per 100,000. After having failed to obtain satisfactory fermentation with a series of pure yeasts, of German origin, it was finally decided to examine the worts, and these were found to be deficient in lime, so that the yeasts grown in them became more and more impoverished in that constituent, as can be seen from the table (vol. ii. p. 144). (The column headed CuO will be dealt with later on.) The yeasts were famished in respect of lime, and even absorbed the small quantities of that base present in the water with which they were washed (vol. ii. p. 86).

H. Seyffert pushed his experiments to the furthest limit, by reducing the already small lime content of the wort still more by dialysis, before pitching it with yeast. In these circumstances a highly frothy fermentation (vol. ii. p. 132) ensued. His observations have also a certain value in connection with the pure culture of yeast, since they demonstrate that a successful result depends not only on the happy selection of a suitable race of yeast, but also on the favourable composition of the nutrient medium—a point already insisted on by HANSEN (III.). Hence, in cases where the application of pure yeast does not fulfil expectations, one should not immediately condemn the innovation. The yeast can only furnish good results when a suitable medium is provided—and this is the task of the practical man.

§ 259.—The Phosphoric Acid Requirements of Yeast.

Yeast requires a good deal of phosphoric acid; and, as can be seen from particulars already given, a considerable amount of this acid is present in the ash. The figures given in the table on p. 141 agree with those reported by K. LINTNER (IV.) as obtained at the Munich Experimental Station, and ranging between the limits of 3.21 and 3.84 per cent. of P_2O_5 (referred to dry matter), the mean being 3.61 per cent. All these values, however, are surpassed by 59.5 per cent. of P_2O_5 found in the ash of English top-fermentation yeast by A. C. SALMON and W. DE VERE MATHEW (I.).

Under ordinary conditions in the brewery the needs of the yeasts for phosphoric acid are satisfied by the phosphates and organic phosphorus compounds present in the malt, though in some cases the amount contained in the barley and malt is inadequate. As a rule, 0.9 per cent., calculated on the dry residue, may be taken as the average phosphoric acid content in barley, though C. LINTNER (I.) reports an instance of a Hungarian barley from the year 1877, which exhibited the remarkable low content of 0.58 per cent. and furnished worts with such a low attenuation, and sedimental yeast of such enfeebled fermentative energy, as to cause great trouble; whereas barleys of the same origin, but from the preceding year, and containing 0.67 to 1.06 per cent. of phosphoric acid, yielded readily fermentable worts under equal conditions. Given parity in the requirements of phosphoric acid content by the yeast, and equal treatment in brewing, a wort low in phosphoric acid will give a relatively far poorer beer. As a matter of fact the corresponding percentages, so far as they have been published up to the present, fluctuate within wide limits, 0.026 and 0.115 per cent. of P_2O_5 in the case of German beers. For this reason, as

Material.	Ash contents in dry residue.	Percentage composition of Ash.									
		K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	CuO.	P_2O_5.	SO_3.	SiO_2.	Cl.
Bavarian wort, according to Lintner . . .	—	41.20	0.03	4.50	2.20	—	—	31.50	trace	20.40	trace
St. Petersburg pale wort, according to Seyffert .	0.24	32.99	1.60	1.63	9.21	0.44	—	45.78	0.32	6.88	0.10
YEAST A:											
1. Obtained direct from Germany . . .	9.71	32.66	0.59	4.48	4.94	0.05	—	55.75	trace	0.51	0.11
2. After being used seven times	8.94	35.22	—	0.54	5.23	0.45	—	56.75	trace	1.22	trace
3. Pure culture, slightly watered	7.56	34.92	—	0.83	5.25	0.29	0.64	56.86	trace	0.80	trace
4. „ „ strongly watered . . .	7.65	31.87	0.07	1.48	5.47	0.42	0.37	58.88	trace	0.98	trace
YEAST B:											
1. Pure culture, used once in wort treated with gypsum, slightly watered	6.82	31.76	trace	3.68	5.38	0.15	0.27	57.67	trace	0·62	trace
2. After being used twice in untreated wort; strongly watered	5.01	29.06	0.14	2.42	5.70	1.21	0.38	60.30	trace	1.21	trace

has already been shown by G. Holzner (I.), the proposal of J. Skalweit (I.) and Fritz Elsner (I.) to employ the phosphoric acid content of beer as a measure of its quality or purity falls to the ground. Lessened attenuation, in consequence of a scarcity of phosphoric acid in the wort, is also frequently experienced in British (top-fermentation) breweries. The remedy applied in such cases is to fortify the wort with phosphates, potassium phosphate in particular. Care is, however, necessary not to employ an overdose, the observations of A. G. Salomon and W. de Vere Mathew (I.) apparently indicating that an excess of phosphates retards fermentation.—Among artificial adjuncts for such purposes, mention may be made of G. Funk and N. von Balogh's (I.) patented method of employing glycerophosphoric acid, $C_3H_5(OH)_2H_2O$, the calcium and magnesium salts of which are soluble in water.

Considerable advantages can be derived from these observations in the preparation of mead or honey wine. Honey is very poor in ash constituents and nitrogenous nutrition, the quantity being usually too small for even moderate development and fermentative activity of the contained yeast cells. The resulting difficulties, well known to all mead manufacturers, can be obviated by treating the honey with nutrient salts. The following recipe for the preparation of mead is based on researches carried out by G. Gastine (I.): About 230 grms. of honey are dissolved in 1 litre of water and treated with 5–7 grms. of a mixture of nutrient salts, composed of diammonium phosphate 100 parts, neutral ammonium tartrate 350, potassium bitartrate 600, magnesia 20, calcium sulphate 50, common salt 3, and tartaric acid 250 parts. The one part of sulphur, also recommended by this author, is, however, preferably omitted. The solution, prepared as above, is boiled up, and after recooling is pitched with wine yeast, which quickly incites a powerful fermentation that runs its due course. It should not be forgotten that an improvement in the flavour of the mead may be expected from the employment of a selected race of yeast. As pointed out by Beyerinck (XVIII.) and others, yeast cells are rarely, if at all, present in the nectar of flowers or natural honey; consequently an artificial addition of high-class yeast is really necessary for obtaining accelerated fermentation, and will be the more successful inasmuch as its action is barely interfered with by the relatively small proportion of other fermentative organisms present. A few observations in this connection have been made by E. Chuard (I)., and more exhaustive experiments by E. Kayser and E. Boullanger (I.). The latter workers also replaced the Gastine nutrient mixture by simpler and equally efficient adjuncts, namely, by treating 1 litre of diluted (24–27 per cent.) honey with either 1.5 c.c. of maltopeptone and 1.5 grms. of potassium tartrate, or with 1.5 c.c. of maltopeptone and 1 grm. of ammonium tartrate; or with 0.12 grm. of spongy peptone, 1.5 grms. of potassium tartrate and 1 grm. of ammonium phosphate. These workers also conducted some experiments in connection with "oenomel," a fermented mixture of honey and wine must.

The opinion expressed by H. Elion (I.) as to the variable requirements of different yeasts in respect of phosphoric acid, and the fluctuations in the resulting increase in fermentative activity, still needs confirmation.

L. Liebermann (I.–III.) asserted that a portion of the phosphoric acid present in the yeast cell is in the form of the meta compound, and, in fact, the same as that contained in nuclein (§ 252), because he believed he had succeeded in isolating barium metaphosphate both directly from yeast, and also from the nucleic acid separated from yeast by himself and B. von Bitto (II.). The analyses advanced in support of this view proved quite as untenable, under the criticism of Kossel (IV.–VI.), as was the case with the cognate characterisation of nuclein as a mixture of the metaphosphates of xanthin and allied bases with a proteid metaphosphate, and the resulting inference that the

artificial nucleins prepared in this way are identical with certain natural nucleins. Nevertheless, the first of these hypotheses has been found accurate, Kossel (V.) himself having detected metaphosphoric acid among the decomposition products of the nucleic acid of yeast (vol. ii. p. 118). Its occurrence was also demonstrated by Alb. Ascoli (I. and II.) in the molecule of plasmic acid, one of the derivatives of that acid. On the other hand, no metaphosphoric acid has been detected in other nuclein bodies, such, for instance, as the paranuclein obtained by the action of pepsin on casein, and in the so-called leuconuclein.

Part of the phosphates or phosphoric acid consumed by yeast is excreted from the latter in the form of phosphocarnic acid. This interesting discovery, which, we are informed by B. Haas (II.), was made by J. Stoklasa, will, if found to be accurate, facilitate the differentiation of natural and sophisticated wines by the chemist, the latter wines usually containing phosphoric acid solely in the form of (added) orthophosphates.

§ 260.—The Importance of Sulphur.

The value of sulphur in connection with the metabolism of yeast is still in complete obscurity. The fact that this substance is never absent in yeast samples justifies the inference that it is indispensable for the growth of the plant. It is almost impossible to prove this directly, *i.e.*, by cultivation experiments, because up to the present no one has succeeded in eliminating the sulphurous impurities (vol. ii. p. 36). from the comparatively large amount of sugar needed to furnish a sufficient crop of yeast for analytical purposes, which impurities—according to a calculation made by Adolf Mayer—suffice to supply the sulphur present in the proteid substances of the crop. The next problem on the list, namely, the nature of the assimilable sulphur compounds taken up by the yeast, also remains unsolved. All that can be said at present is that the sulphates (of calcium and magnesium), so greatly appreciated by the higher plants, appear ill adapted for the construction of the yeast cell. The sulphur in these salts is eliminated and expelled either as sulphur dioxide or even sulphuretted hydrogen. Further information on this point will no doubt be welcomed by fermentation technologists.

The first reliable information on the production of sulphur dioxide during alcoholic fermentation by yeast was supplied by Fr. Pfeifer (I.), who traced the gradual accumulation of this reduction product in fermenting beer wort, and obtained the following figures:

SO_2 Content in Mgrms. per Litre.

	Lager Beer.	Draught Beer.	
Wort from the filter-bag . . .	traces	traces	traces
At the beginning of fermentation . .	11.1	7.6	2.9
In cask, after close of primary fermentation	11.7	8.8	3.7
After storage for a month . . .	12.5	7.7	?
On leaving the brewery . . .	?	9.6	4.7

In saccharose solutions, treated with the necessary nutrient salts (including ammonium sulphate), sterilised and inoculated with a large quantity of yeast, 11.4 mgrms. of SO_2 were detected at the end of five days, when fermentation was almost completed. The same results were obtained by B. Haas (I.) in his experiments with fermenting wine must, though he raised the point that the

collaboration of reducing bacteria was not impossible. In a fermenting must, seven weeks old, he found 49.4 mgrms. of SO_2 per litre; and two months later 576 mgrms. In view, however, of the determinations of Pfeifer, confirmed by the observations of S. Klaudi and A. Svoboda (I.), his statement that the reduction of the sulphates occurs only with thin sowings of yeast and sluggish fermentation cannot be accepted. E. Hotter (I.) also found 4.5–4.8 mgrms. of SO_2 per litre in cider and currant wines prepared in the laboratory, and certainly not sulphured.

These discoveries have no small importance for the fermentation technologist and foodstuff chemist, since, until recently, it was usual to consider that beer found to contain sulphur dioxide must have been made from strongly sulphured hops, or else treated with calcium sulphite as a preservative. Similar conclusions (cask sulphuring or washing with calcium bisulphite) were also formed with regard to wines found to contain sulphur, more especially since L. Roesler (I.) in 1885 stated that he had never succeeded in detecting sulphur dioxide in wine prepared in the laboratory so as to preclude these sources of sulphur. The present state of our knowledge shows that the matter is different and that great care must be exercised, in this direction also, in judging the results of analysis, a schooling in fermentation physiology being moreover indispensable. Both the sulphur dioxide produced during fermentation and that originating in the sulphuring of the casks is almost entirely converted into a state of combination during the storage of the wine (§ 79), so that, as was confirmed by M. Ripper (I.) and R. Kayser (I.), only extremely minute quantities of free SO_2, mostly inferior to 2 mgrms. per 100 c.c., are present in wine that is ready for bottling. This free dioxide alone comes under consideration in judging wine from the medico-physiological standpoint, and not that present as aldehydic sulphur dioxide, which is not merely innocuous to health—according to the researches of J. Marischler (I.)—but really essential to the bouquet of the wine. In consequence of the reducing power of the dioxide, the presence of larger quantities in beer or wine affects the results of sugar determinations with Fehling's solution, causing, as was first pointed out by Jos. Herz (I.), the sugar values to come out in excess of the truth.

In certain circumstances the reduction of the sulphates in the nutrient solution by the activity of yeast may proceed a stage further than the formation of the dioxide, namely, to the production of sulphuretted hydrogen. However, the first report on this point, as made by Crouzel (I.), was found inaccurate when tested by F. Gay (I.), though confirmed by the researches of Nastukoff (I.) with pure cultures. A solution of 10 per cent. of saccharose and 0.5 per cent. of Gastine's nutrient salt mixture (§ 259), in which the calcium sulphate had been replaced by magnesium sulphate, was inoculated with pure wine yeasts from Portugal and Champagne, Brussels beer yeast, *Saccharomyces apiculatus* and *Sacch. Pastorianus*, all of which yeasts proved capable of producing the gas in question. The question, arising from this result, as to the dependence of the reduction phenomenon on the environment of the culture, was more closely examined by A. L. Stern (I.), who failed to discover any substance of *known* constitution capable of supplying the yeast with sulphur without the concurrent liberation of sulphuretted hydrogen. This agrees well with the fact, known to every chemist and fermentation technologist and first pointed out by Reischauer, that the distillates from beer very frequently contain sulphuretted hydrogen, or a compound exhibiting all the characteristics of the same.

Moreover, the formation of sulphuretted hydrogen by the reducing action of yeast is not confined to the sulphates; even sulphur itself may serve by combining with the hydrogen liberated from other substances by the activity of a yeast enzyme (philothion), which will be dealt with, in conjunction with other allied matters, in a subsequent chapter.

CHAPTER L.

ORGANIC FOODSTUFFS. THE REQUIREMENTS IN RESPECT OF OXYGEN.

§ 261.—Sources of Carbon.

The true water content, and therefore also the amount of dry residue (dry matter) in the yeast cell itself, is not yet accurately known. The figures cited in the literature are based on experiments performed, not on the cells only, but on samples of sedimental yeast from the brewery, or pressed yeast, neither of which, as we have already seen (vol. ii. pp. 85, 128, 129), is of uniform nature, but includes a variety of organic and inorganic admixtures. The amount of dry residue in pressed yeast is determined, of course, by the amount of pressure employed. R. Kusserow (III.), in testing eight different samples, free from starch, found 22.1 per cent. as the minimum, 29.9 per cent. as the maximum, and 25.6 per cent. as the average water content. In practice, 26 per cent. is usually estimated.

The sp. gr. of the cells of pressed yeast was determined as 1.1 by P. Guichard (I.) in 1894, though the method, suspension of the cells in a mixture of alcohol and chloroform, was not perfectly reliable, and probably gave results in excess of the truth. On the other hand, the pycnometric method adopted by Kusserow for determining the sp. gr. of his eight samples of pressed yeast, gives values that are probably too low. He weighs out exactly 10 grms. of the yeast, triturates them with a little distilled water in a porcelain basin, and swills the mixture into a pycnometer, which is then filled up to the mark with distilled water and weighed. Taking O as the weight of the whole and P the weight of the pycnometer filled with water alone, the difference O-P being equal to a, the sp. gr. of the sample of pressed yeast works out to $S = 10 : 10 - a$. Kusserow determined the maximum value as 1.1093 and the minimum as 1.0821.

The sp. gr. of the dry residue of these samples ranged from 1.580 and 1.491, with 1.509 as the mean value. By assuming (which is not strictly accurate) that the volume of the yeast sample is equal to that of the percentage, by weight (T), of the dry residue, plus that of the water content (W), and taking the above mean into consideration, we obtain the equation

$$\frac{W}{1} + \frac{T}{1.509} = \frac{100}{S},$$

and, the sp. gr. of the sample being known, this gives the equation

$$T = 296.5 \left(1 - \frac{1}{S}\right),$$

whilst, the dry residue being known, the sp. gr. of the yeast sample is found by the equation $S = 296.5 : (296.5 - T)$.

Contrary to the proposition advanced by Hayduck, however, it is unfortunately impracticable to determine the quantitative addition of starch in a sample of pressed yeast by this method, the difference between the sp. gr. of anhydrous starch (mean 1.65) and that of the dry residue of the yeast (1.509)

being too small. This method is discarded with greater regret because the existing chemical methods, based essentially on the hydrolysis of the starch and the determination of the resulting sugar, are very unreliable owing to the fact that this treatment saccharifies the glycogen of the yeast as well as the added starch, and that the amount of the former (vol. ii. p. 124) is sometimes very large, occasionally exceeding that of the starch itself.

The ultimate composition of the organic matter in the dry residue of yeast cells is influenced by the mode of nutrition as well as by the kind and age of the cells, for which reason generalised values are unreliable. Moreover, the available analytical data on this matter have not been obtained by working with actual cells, but from the examination of pitching yeast or pressed yeast. Now the invariable presence of admixtures in these samples, already alluded to in § 257 as preventing the acquisition of reliable data on the ash constituents of the yeast cell, has a still greater adverse influence when the determination of the amount of carbon, hydrogen and oxygen in the cells is in question, many of these admixtures being low in or free from ash, and consisting solely of three or four of the elements just mentioned. Consequently the results of the ultimate analysis may differ between wide limits, according to the proportion of such impurities present. As instances of this, and not merely to comply with an injudicious demand for quantitative reports on the ultimate composition of yeast, a few results obtained in this connection are reproduced below. Although on the publication of the first analysis by Marcet the useless character of such figures was pointed out by Quevenne (I.) in 1838, similar results have been brought forward from time to time since. In fact, some workers have gone so far as to assume that an expression of the difference between top and bottom yeasts can be found in the results furnished by ultimate analysis—a view that is, of course, untenable.

Carbon compounds may be taken up by yeast for three purposes: (1) for alcoholic fermentation and other enzyme actions; (2) to replace the energy dissipated by respiration; (3) for the formation of new cell substance in growing cells, or to replace constituents decomposed and excreted, in consequence of other metabolic changes in full-grown cells. The first of these three causes of the consumption of carbon compounds will be discussed thoroughly in a subsequent section, and will therefore be omitted from the present paragraph. The second has also been referred to on pp. 91, 92 of vol. ii., and will be supplemented later; so that we have at present only the third to deal with.

We are indebted more particularly to Emil Laurent (VI.) for a comprehensive investigation of a large number of carbon compounds in respect of their

Ultimate Percentage Composition of the Organic Matter of Yeast Referred to Dry Residue free from Ash.

Author.	Class of Yeast.	C	H	N	O	S	P
Marcet	Beer yeast	30.5	4.5	7.6		45.4	
Dumas (I.) . . .	„ „	50.6	7.3	15.0	—	27.1	—
Mitscherlich (III.) .	„ „	47.0	6.6	10.0	?	0.6	—
Schlossberger (I.) .	„ (top yeast)	49.8	6.7	12.4	31.1	—	—
Hessenland (I.) . .	„ „	48.6	7.1	7.8	36.6	—	—
„	bottom yeast	49.3	8.2	10.5	32.0	—	—

suitability for supplying yeast with carbon. These researches were performed on a series of pure culture beer and wine yeasts, with both mineral nutrient solutions and gelatin nutrient media, and showed that the following substances

can be absorbed and assimilated as sources of carbon: the acetates of potassium, sodium and ammonium; lactic acid and the lactates of these three bases and of calcium; malonic acid and its potassium salt; succinic acid and its ammonium salt; the potassium and calcium salts of glyceric acid; the calcium salt of glycerophosphoric acid; malic acid and its potassium and ammonium salts; dextro-tartaric acid and its potassium and ammonium salts; levo-tartaric acid; citric acid and its potassium and ammonium salts; mucinic acid; fumaric acid; aspartic acid; asparagin; glutaminic acid (all in the proportion of 1 per cent.); glycerin, mannitol, quercitol, glucose, fructose, saccharose, maltose, lactose, dextrin, salicin, amygdalin, and many others. On the other hand, the following were not assimilated by the yeasts (in sedimental and not film cultures): methyl-, ethyl-, propyl-, and butyl-alcohol (2–4 per cent.), formic acid and its potassium, sodium, ammonium and calcium salts, acetic acid, propionic acid and its potassium salt, butyric acid, valerianic acid, stearic acid, oleic acid and its potassium salt, sodium butyrate, oxalic acid and its potassium and ammonium salts; the ammonium salts of benzoic acid, salicylic acid, and gallic acid; urea (all as 1 per cent. additions).

When yeast is cultivated as film cultures on the surface of the nutrient solution, and not under the conditions employed by Laurent, it is also capable of utilising alcohol, though chiefly, or even exclusively, by respiration, a method we are not considering at present.

Both in nature and in the practice of the fermentation industries, the carbohydrates form the usual and preferential material from which yeast obtains its requirements in respect of carbon, the chief part in this respect being played by certain sugars. The behaviour of yeast toward these latter, with regard to their assimilation as distinct from fermentation, has not, however, been sufficiently investigated, the results of Laurent's researches on this point being unsuitable for generalisation, since they apply solely to the species of yeast tested by him, and not to all the others. We are indebted to Beyerinck (XVIII.) for the discovery that the *Schizosaccharomyces octosporus*, found by him on currants, forms an exception to Laurent's rule, inasmuch as it is capable of assimilating maltose, glucose and fructose, but not saccharose, lactose, raffinose, arabinose, dulcitol, quercitol, erythritol and inositol. The antithesis of this species is *Saccharomyces Zopfii*, which, according to Artari (I.), can cover its needs in respect of carbon from saccharose, glucose and mannitol, but not from maltose, lactose, galactose, inulose or melampyrit. Similar behaviour to the last-named organism is afforded by a yeast discovered by Beyerinck (XXI.), and named by him *Saccharomyces fragrans* on account of the fragrant ester it produces. *Sacch. kefir* and Beyerinck's *Sacch. acetethylicus* assimilate glucose, fructose, maltose and saccharose, the last-named one utilising lactose as well. With regard to the suitability of this last disaccharide as a source of carbon, P. Mazé (I.) experimented with eleven stocks of yeast from soft cheese. These few examples will show that, also in respect of assimilation, the only way to obtain really applicable results is by working with pure cultures; and it is owing to the omission of this essential condition that both the experiments of C. von Nägeli (IV.) in the "seventies," and the publications of T. Bokorny and other workers must be left out of consideration here. Dextrin seems to form a good source of carbon for most yeasts; in Beyerinck's (XXI.) experiments it was only refused by a single species.

The suitability of the pentoses ($C_5H_{10}O_5$) as sources of carbon for yeast has not yet been examined by fermentation physiologists as thoroughly as might be desired in the interest of the fermentation industries. The parent substances of these sugars, namely, the pentosans ($C_5H_8O_4$) occur in abundance as an important constituent of the vegetable cell-wall in cereals as well, B. Tollens and

H. Glaubitz (I.) having found up to 8.9 per cent. (based on dry matter) in barley, 11.2 per cent. in malt, 8.7 per cent. in wheat, 11.1 per cent. in rye, and 5.8 per cent. in maize. The question whether and in what proportions the pentosans of barley undergo hydrolysis during germination has not been exactly determined, even in the exhaustive researches of Cross, Bevan, and Cl. Smith (I.), though it is certain that this occurs during the kilning of malt, varying amounts of furfural being produced, according to the working conditions. Tollens and Glaubitz state that about three-fourths of the pentosans of the malt are left in the grains by the mashing process in the brewery, partly, however, no longer in the form of pentosans, but as the resulting pentoses. These, though unfermentable (*see* chapter lxix.), may serve as sources of carbon for the yeast, provided the external conditions be favourable, as in the case of certain experiments conducted by H. van Laer (I.) and by Cross and Bevan (I). In other cases, however, as shown by Beyerinck (XXI.) with regard to arabinose in the case of *Schizosacch. octosporus*, they are utilised to only a small extent or not at all. Pentoses are formed, in still larger quantity than in brewing, in raw-grain distillery mashes, where the raw grain is dissociated by steaming for several hours under a pressure of 3–4 atmospheres.

Beyerinck's (XVIII.) proposal to divide the genus *Saccharomyces* into six sub-genera: *Glucomyces*, *Maltomyces*, *Lactomyces*, *Raffinomyces*, *Polysaccharomyces* and *Dextrinomyces*, in accordance with their characteristic behaviour toward the various carbohydrates, is scarcely feasible (*see* chapter ix.).

When more than one assimilable source of carbon is present in the nutrient medium, selective power (*see* p. 36, vol. i.) is exercised. In yeasts the study of this property—so far as sugars are concerned—is very difficult, and little progress has been made, because of the intervention of fermentative action in most cases, so that the separate determination of the amount of sugar consumed for the structural purposes of the cell cannot be performed with sufficient accuracy, if at all. A great influence on the ratio of the quantities of two or more nutrient substances in unit time is exercised by their relative diffusibility, this being also determinative when two or more fermentable sugars are at the disposal of, and being fermented by, the yeast. The selective power of yeast in fermentation has already been accurately tested, but the results must be postponed to chapter lxix., where also the extensive literature on selective fermentation will be cited. At present we can only take into consideration the discovery that, when two or more diffusible carbohydrates are present, the one exercising the greater osmotic pressure will diffuse more abundantly in the cell per unit of time, glucose, for instance, more than fructose. According to E. Prior and H. Schulze (I.), the permeability of the cell membrane varies in the several species of yeast.

The amount of carbohydrates consumed in the formation of cells depends on the rate of reproduction, and therefore on the nature and extent of the influences controlling same. Pasteur (VII.), for a series of experiments, calculated the consumption for this purpose to be about 1 per cent. of the total saccharose consumed; and Balling (I.) stated that 5.323 parts of dry matter of yeast are formed for every 100 parts of wort extract (not only carbohydrates) disappearing in primary fermentation during the reproduction of bottom-fermentation yeast. In an experiment by Giltay and Aberson (II.), one part of yeast was obtained for every 3.8 parts of the total sugar consumed.

§ 262.—Inorganic Sources of Nitrogen.

When, in the course of his controversy with Liebig on the character of alcoholic fermentation, and on the nature of yeast as a living organism (*see*

p. 94, vol. i.), Pasteur (XXIII.), in 1858, made his victorious discovery that this ferment is also active in a solution containing nitrogen solely in the form of ammonium tartrate, the term "yeast" was still very vague, the question whether wine yeast or beer yeast consisted of several species of organisms probably differing considerably in their foodstuff requirements had not come up for discussion, and there was no reliable means available for separating such a mixture of species into its components, and then examining the latter separately. Hence no clear light could be thrown on the matter by argument on the point of these observations. Liebig's (II.) statement in 1869 that he failed to obtain either fermentation or reproduction of the sowing, in an accurate repetition of Pasteur's experiment, was, in the opinion of the latter (XXIV.), sufficiently disposed of by the offer to perform the experiment again in the presence of any trustworthy person appointed by his opponent, and produce as much yeast as the latter "could reasonably desire." An objection urged by Millon (II). was controverted in 1864 by Duclaux (XVI.), and by degrees Pasteur's assumption that yeast is able to satisfy its nitrogen requirements from inorganic sources exclusively, assumed the position of an unassailable law, observations to the contrary being reported with diffidence. A. Mayer (I.), whose researches on the nitrogen requirement of yeast in 1869 led him to adopt substantially the same opinion as Pasteur, observed—as did also the latter, and subsequently Nägeli (IV.) as well—that "the nutrition of yeast at the expense of ammonium salts always proceeds with somewhat greater difficulty than with nitrogenous yeast extract," and added, "in the former case *a larger number of well-organised yeast elements are required* to induce fermentation." The limitation implied by the words italicised was not determined until twenty-two years later.

Wildiers (I.), in 1901, was the first to show, by the use of pure cultures of top-fermentation beer yeast of the *Sacch. cerevisiæ I.*, Hansen type, that neither fermentation nor yeast reproduction took place in 125 c.c. of a saccharified nutrient solution of mineral salts, when only a very small number of yeast cells were used for inoculation, *e.g.*, about as many as are contained in two drops of a culture grown in beer wort, or in 0.25–1.0 c.c. of a mixture of pressed yeast with ten parts of water. On the other hand, both fermentation and reproduction took place when the inoculation was accompanied by the addition of a few c.c. of a decoction of yeast, or of Liebig's meat extract, peptone or wort. From these observations Wildiers concluded that nitrogen in inorganic combination is insufficient for the needs of the yeast cell, the growth and fermentation also requiring a certain quantity of a special unknown substance, absent from inorganic foodstuffs, and which he proposed to term "Bios" (Gr. = Life). This substance is not an ash constituent; it is destroyed (rendered inactive) by boiling in 20 per cent. sulphuric acid, can be dialysed, is soluble in water, and can be extracted with this solvent from yeast (especially on boiling). Yeast, though containing bios, is incapable of elaborating it; so that when a small amount of yeast is taken for inoculation, the quantity of bios introduced into the mineral nutrient solution is insufficient for reproduction, whereas with a more plentiful inoculation enough is introduced to allow new cells to be formed at the expense of such as are moribund.

Owing to their highly important bearing on the study of the nutrition of yeast, these observations deserve a thorough experimental investigation; but at the outset they were yearly subjected to deprecatory criticism, as being opposed to the ruling dogma. Some asserted that the dependence of the result of the experiments on the amount of the inoculation was due to the presence, in Wildiers' nutrient solutions, of poisons, such as copper, derived (in traces) from the distilled water or present in the air of the laboratory, or ultramarine contained

in the commercial saccharose used in the experiments, although Wildiers expressly stated that no difference in the results was obtained by working with invert sugar. To bios was ascribed the task of rendering these poisons innocuous, becoming thereby itself inactive and unsuitable as a yeast food, the further quantities, present only in larger sowings, being required for the needs of the cells. This opinion was tested, in a series of exhaustive experiments, by A. Amand (I.), who showed that bios does not play the part of an antidote.

One of the next measures was to obtain quantitative data on the new problem. Wildiers mainly judged the results of his experiments by the quantity of carbon dioxide liberated from the cultures, and did not make any exact determinations on the number of cells sown and gathered. This, it should be distinctly observed, is a factor that does not affect the principle of the question; but in later researches it was impossible to forget, in the investigation of the conditions of cell reproduction, the quantity of the matter influenced, as well as the dimensions of the influence. This requirement was first satisfied by Al. Kossowicz (I.) in 1903. This worker, operating with pure cultures of *Sacch. ellipsoideus I.* (Hansen) and the distillery yeast, Race II. of the Berlin Experimental Station, found, for instance, that 200 cells of the former yeast sown in 100 c.c. of saccharified mineral nutrient solution increased to 140 million cells in fifty days. Subsequently (II.) he extended the work by sowing single cells; but in twenty-one out of twenty-two tests no development at all could be detected under the microscope, and the only positive result (which was very scanty) was probably due to the accidental introduction of a larger quantity of wort-gelatin along with the cell used for inoculation.

According to the observations of A. Amand (II.), the amount of bios in the nutrient solutions sown with yeast decreases very rapidly, and can then no longer be demonstrated in the cells, at least by the lixiviation method. J. Henry (I.), on the contrary, believed he had found yeast capable of forming new quantities of bios, a capacity certainly possessed by other fungi (*Penicillium glaucum* and a *Mycoderma*), as was first demonstrated by Kossowicz (I.). Thus, saccharified mineral nutrient solutions in which no development took place, owing to insufficient sowings (in parallel tests), gave both reproduction and fermentation on the *Eumycetes* in question being sown along with the yeast or had been previously grown in the solution and then killed off by heat before the introduction of the yeast. This observation, subsequently confirmed by A. Amand (II.), is valuable in connection with the interpretation of the results of previous workers. Commercial pressed yeast and wine yeast are almost always contaminated with *Mycoderma*, and the same is often the case with brewers' pitching yeast. These *Mycoderma*, however, as shown by Winogradsky (XI.) and Kossowicz (with different species), even in the case of rapid reproduction from a small sowing, are able to satisfy their nitrogen requirements from ammonium salts exclusively. Hence, when introduced with a sowing of yeast into mineral nutrient solutions, they develop first, in spite of their originally minute number, and then prepare the nutrient medium in the above sense for the purposes of the hitherto quiescent yeast. Ad. Mayer (I.) also reports that in his experiments (already mentioned on the previous page), "*Mycoderma vini*" almost invariably appeared. Hence, as is now admitted, his results do not apply solely to yeast, and show that the only way to obtain reliable data regarding the nitrogenous nutriment of yeast is by the aid of pure cultures, no others being worth the trouble of undertaking.

The problem set out above is still too fresh for a final solution to have been found; and each day may reveal some new observation opening up a quite unexpected perspective; for which reason the matter has been very briefly treated here. However, one point is now well established, namely, that in a saccharified nutrient mineral solution containing ammonia as the sole form of nitrogen

cell reproduction and fermentation can only commence when the number of cells introduced does not fall below a certain minimum, the absolute dimensions of which have not yet been definitely ascertained, and which is probably dependent on the other conditions of the experiment.

This, however, does not imply that yeasts unconditionally reject ammonium salts when the other conditions essential to development are present. On the contrary, they then exhibit a certain preference for ammoniacal nitrogen. This was already observed by DUCLAUX (XVII.) during his experiments on the fermentation of wine must in 1866, the nitrogen content falling from 120 mgrms. to a very small proportion per litre in consequence of its consumption by the yeast present. The question has also been studied by Müntz and Rousseaux, then by Roos and Chabert, and finally by J. Laborde, the last-named stating that, at 28° C., ammoniacal nitrogen is taken up more extensively than organic nitrogen, though the reverse was found to occur at 36° C. According to ARTARI (I.), *Sacch. Zopfii* is even satisfied with ammonium sulphate as the sole source of nitrogen. Further particulars respecting the behaviour of *Mycoderma* species toward ammonia salts will be given in chapter lx.

The nitrates, which are the best, and in some cases the only, sources of nitrogen for higher plants, are of no value for yeasts, except in a few cases, typified by BEYERINCK'S (XXI.) *Sacch. acetethylicus*. This was first proved by AD. MAYER (I.); and the converse opinion expressed by DUBRUNFANT (III.), was disproved by E. LAURENT (VI.). The injurious effect produced on yeast by the presence of such salts in otherwise favourable nutrient solutions seems due to the reducing action of the cells causing the formation of highly poisonous nitrites. One of the reasons of fermentation disturbances in molasses distilleries is certainly to be found in the presence of nitrates, which sometimes occur in large quantities in molasses. Similar results have also been found by L. BRIANT (II.) in breweries employing water rich in nitrates; and this worker mentions about 75 grains per gallon as the highest permissible limit of these salts. The influence of nitrates on attenuation has also formed the subject of experiments by EVANS (I.). In a patented process for cultivating races of yeast capable of thoroughly fermenting dextrins (*see* chapter lxv.), J. EFFRONT (VIII.) is said to employ a nutrient medium containing nitrates as the exclusive source of nitrogen.

§ 263.—Organic Sources of Nitrogen.

In the worts, mashes, and musts used in practical fermentation, the yeast has not to depend on inorganic sources of nitrogen, but generally has at its disposal an abundance of readily assimilable organic nitrogen compounds. The suitability of a few representatives of this large class will be more closely considered in the following paragraphs.

Among the amides suitable as nitrogenous foodstuffs, special attention is merited by the acid amide of aspartic acid

$$(COOH—CH_2—CH.NH_2—COOH)$$

namely, asparagin

$$(COOH—CH_2—CH.NH_2—CO.NH_2),$$

this substance playing an important part in the mashes used in practice. It is always formed during the germination of seeds, and is therefore present in malt, and still more abundantly in malt culms. Even potatoes contain appreciable quantities. Finally, in addition to other amides, asparagin is one of the chief forms in which nitrogen occurs in the molasses of beet sugar works; proteins, on the other hand, being almost entirely excluded or eliminated by the method employed for obtaining the juice from the beet in the diffuser and

by the purification process in the saturator. M. Hayduck (IV.) recognised asparagin as an excellent source of nitrogen in the nutrition of yeast; and this was confirmed by E. Laurent (VI.), G. Heinzelmann (IV.), H. P. Wijsmann (II.), and others. Yeast is capable of transforming asparagin into proteins, a property unshared by the animal organism so far as is known at present. The nitrogen of asparagin (which is worthless as a food for animals) is present in the potatoes made into distillery mash, is recovered in the distillery waste in the form of protein, and imparts to this waste product the character of a concentrated fodder for stall-fed cattle. In an experiment carried out by P. Petit (II.) with a nutrient medium containing asparagin and ammonium phosphate, it was found that top-fermentation yeast consumed twice as much of the former as was utilised by bottom yeast—a difference considered by the author to afford a means of differentiating these two yeasts. According to a comparison instituted by R. Kusserow (I.), on the relative influence of asparagin and peptone as the source of nitrogen in saccharified mineral nutrient solutions, the former substance accelerates fermentation and increases the yeast crop. The observation that when grown with the aid of asparagin, the cells of the sedimental yeast are not cohesive, whereas in the case of peptone they unite into flocculent masses, which settle down less firmly than the others, was also confirmed by H. Lange (I.) with beer wort, his explanation being that the cohesion is due to precipitated peptone. A high selective power is exerted by *Schizosacch. octosporus*, which, according to Beyerinck (XVIII.) is suited only by the nitrogen compounds naturally present in raisins or malt, and not by ammonium salts, asparagin or peptone.

Among cereal grains, rye is characterised by a high percentage of proteins specially suitable for the formation of yeast protoplasm. This is one of the reasons why yeast manufacturers, particularly those working with the old (Vienna) method, prepare their mashes with an addition (usually one-third) of this grain. Its percentage content of nitrogenous substances fluctuates, however, between wide limits, 7.5 per cent. on the one hand and 15.3 per cent. on the other, being by no means exceptional figures—as was shown by Delbrück (IV.) in a highly interesting experiment. Manufacturers endeavour to counteract the resulting great variation in the amount of the yeast crop by employing mixtures of rye of different origin; but they would, no doubt, prefer to be able to determine the amount of these proteins in buying the raw material, and to value the latter accordingly. On this point, however, there are no data at present available. Among the proteins and allied substances mention should also be made of diastase, which is not merely capable of serving as a source of nitrogen when present as the sole nitrogenous constituent of the nutrient medium, but is also taken up and utilised by the yeast, and thereby caused to disappear, even when peptone and asparagin are also present in sufficient quantity. This observation, recorded by Heinzelmann (IV.), is of interest in connection with the fermentation of raw grain (maize, &c.) mashes, in which the unsaccharified dextrin is intended to be hydrolysed by diastase during the prolonged primary fermentation. In one experiment performed by Heinzelmann (V.), out of the 37 parts of diastase left from each 100 originally present at the end of the mashing process, 33 4 disappeared during fermentation, so that only 3.6 remained in the fermented mash.

When amides are present in nutrient media together with proteins and their nearest degradation products, the selective power of the cells (*see* p. 36, vol. i.) becomes manifest. In this connection a number of very instructive data are already available. The degradation products of protein are always present in brewery and distillery mashes, owing to the activity of the proteolytic enzymes in the malt used. In an experiment performed by C. J. Lintner (V.), out of the total nitrogen (0.092 per cent.) in the saccharified malt extract used as the

nutrient medium, and composed of 0.062 per cent. of amides and 0.030 per cent. of proteins, &c., 0.036 per cent. (viz., 0.030 per cent. of amide nitrogen and only 0.008 per cent. of proteid nitrogen) was absorbed by the yeast. Wahl and Hantke (I.) found the relative proportions of nitrogen absorbed (from wort) by the yeast, in the form of proteins, peptone and amides, were: 0.4 : 1.7 : 19.9 mgrms. respectively per 1000 c.c., the initial content in the wort being 9.0 : 27.4 : 52.0. In this case also amides were preferentially absorbed. Though P. Petit and G. Labourasse (I.) consider that their observations justify an opposite conclusion, it follows from R. Kusserow's (IV.) experiments that the protein degradation products are better foodstuffs than the unaltered proteins. This partly explains the favourable influence of an addition of malt culms to the mash, especially in cases of sluggish fermentation of rich mashes. This throws new light on the influence of the kind of mashing process on the protein content of beer wort, and thereby on the progress of the development of yeast, as also the course of fermentation and the character of the resulting beer, especially in contrasting the two opposite processes of the infusion method on the one hand and the Bavarian thick-mash method on the other. Adalbert Flühler (I.) was the first to draw attention to this, and his work was continued by V. Griessmayer (II.). C. J. Lintner (V.) reported that these discoveries were confirmed by Ad. Ott.

Hayduck (IV.) carried out the first experiments worthy of mention on the ratio between the size of the yeast crop and the amount of the nitrogen content in the nutrient medium; and this worker found that asparagin, when present as the sole source of nitrogen, and to an extent not exceeding 0.25 per cent., in a saccharified solution of mineral salts, was completely absorbed by the added pressed yeast. With a larger quantity of asparagin present, more was consumed, but in all cases a portion remained undecomposed. On the other hand, A. L. Stern (I. and III.) found 0 025 per cent. of asparagin to be the maximum, no appreciable increase in the reproduction of the cells occurring with any larger quantity. A similar observation was made by P. Thomas (I.) with regard to urea as a source of nitrogen. Iwanowski (II.) states that the degree of alcoholic fermentation in a saccharified mineral-salt solution varies inversely with the amount of peptone added as the source of nitrogen. On the other hand, in experiments with wine must containing 1 per cent. of added peptone, J. Behrens (XIII.) found that this addition assisted fermentation (by pure yeast).

The observations of D. Delbrück (IV.), Ces. Forti (I.) and E. Boullanger (I.) show that the quantity of nitrogen absorbed from the nutrient medium is primarily dependent on the species of yeast employed. It also varies, however, with one and the same yeast, according to the other conditions of environment, being greater, for instance, in aerated and strongly agitated cultures. This was noted both by the last-named worker and also by C. F. Hyde (I.) who found that 23.8 per cent. of the nitrogen originally present in a given wort was eliminated by means of a large sowing of yeast, coupled with rousing and agitation, whereas with a medium sowing without aeration only 17.2 per cent. was removed, and merely 15.8 per cent. with a small sowing and slight aeration.

The influence of the nature of the source of nitrogen is revealed by the observation made by A. L. Stern (V.) that in parallel cultures with equal initial nitrogen content, either in the form of asparagin, peptone or yeast extract, the relative quantities of this element eliminated by the yeast were 1 : 1.8 : 2.2. That the amount of sugar present in the nutrient medium also influences the absorption of nitrogen has been shown by P. Thomas (I.) and also by Stern (III.) The former observer found that a larger quantity of the urea offered as the source of nitrogen was assimilated in presence of 20 per cent. of dextrose than when only 10 per cent. of that sugar was available. In Stern's experiments the largest

quantity of asparagin was assimilated from a mineral-salt solution containing 0.3 per cent. of that substance when the added dextrose amounted to 15 per cent. (the limits ranging from 0 to 30 per cent.); but when only 0.15 per cent. of asparagin was present the optimum quantity of sugar fell to 12.5 per cent.

Apart from the exceptions to be mentioned hereafter, all the worts, musts, and mashes fermented on a practical scale contain a surplus of nitrogenous nutriment; so that this is still far from being exhausted by the time cell reproduction has come to a standstill in consequence of the gradual change for the worse in the other conditions of nutrition, especially by the increased alcohol content (*see* chapter lii.). Hence a larger or smaller quantity of useful nitrogenous nutriment remains in the fermented product. As we have been informed by J. von Liebig (II.), Graham, A. W. Hoffmann, and Redwood in 1853 found that pale English worts containing 0.217 per cent. of nitrogen furnished beers containing 0.134 per cent. Similar investigations—also with infusion worts in an English brewery—were made by H. Grimmer (I.), with the result that, of the nitrogen (0.132–0.138 per cent.) in the original wort, about one-fourth (24–26 per cent.) was found to have been taken up by the yeast, the greater portion (one-half to two-thirds) being absorbed during the first twenty to twenty-four hours after pitching. This was confirmed by C. F. Hyde (I.). Substantially the same observation was made by Delbrück (I.) in the case of pressed yeast in 1879, so that the nitrogen consumption curve rises very sharply. One pressed yeast and three different samples of low-fermentation beer yeast (containing 8.24 and 8.94 to 9.54 per cent. of nitrogen in the dry residue), which were allowed by Hayduck (V., VI.) to develop under identical conditions in malt-extract solution containing initially 0.0876 per cent. of nitrogen, absorbed 43 and 30–39 per cent. of this foodstuff for the structural purposes of the cell. That the residual nitrogenous substances in young beer are capable of affording nutrition for the further development of yeast has been repeatedly shown, *inter alia*, by F. Hyde (I.) and Hayduck (V., VI.), though it would be erroneous to assume that the whole of these nitrogenous substances are suitable for this purpose, or that any one of them is accessible to any race of yeast. Unfortunately, the observations available on this important question are but few, *e.g.*, those communicated by Delbrück (V.). The appearance of yeasty haze in lager beer (*see* p. 136, vol. ii.) and in stored wine may probably be traced by further investigation to wort or must proteids, which the yeast concerned in primary fermentation has been unable to consume, but which during storage has furnished structural material for the development of some other race of yeast that has crept in in the meantime. Wine must also contains an excess of nitrogenous nutriment for the yeast, and left unconsumed by the latter. H. Müller-Thurgau (II.) took a Geisenheimer Riesling must of 1888 vintage, which he subjected to six fermentations in succession, removing the resulting yeast and alcohol and adding fresh sugar each time, the final result being a wine containing 0.051 grm. of nitrogen per 100 c.c., as against 0.100 grm. in the must, whilst the removed yeast contained 0.049 grm. In accordance with the gradual diminution of nitrogen in the maturing wine, the successive deposits of yeast separated from the supernatant wine also exhibit a diminished nitrogen content. Thus in a case examined by A. Czéh and H. Müller-Thurgau (I.), the first deposit of yeast furnished 6.19 per cent., and the fourth only 4.3 per cent. The course of fermentation in the must is also influenced by the method of treating the vines. Müller-Thurgau (II.) reported on a case of stormy fermentation in must which, in consequence of the heavy manuring of the vineyard, contained not less than 0.12 per cent. of nitrogen. The residue of nitrogenous constituents in the new wine, after the removal of the primary yeast, enables the secondary fermentation to be carried through.

Apple and pear musts are frequently poor in nitrogenous yeast food, and this is generally the case with berry musts, especially bilberry must; and for this reason they ferment very sluggishly and incompletely. These defects may be remedied—as was first advocated by Nessler and tried by H. Müller-Thurgau—by an addition of 20 grms. of sal ammoniac per hectolitre (about 3 oz. per 100 gallons). Similar results, but at higher cost, can be obtained—as was done by R. Otto (I.)—by the use of ammonium tartrate or even asparagin.

It would be incorrect to suppose that the total quantity of nitrogenous matters present in wines or beers already existed as such in the must, wort, or mash. On the contrary, a portion, varying considerably according to the conditions of fermentation, originates in the metabolism of the yeast employed, and is excreted by the latter; if soluble, it remains in the liquid, but if transformed into the insoluble condition it will be found in the sedimental yeast. The quantity of the soluble matters excreted from the cells increases with the temperature, other conditions being equal. The experiments of E. Hantke (I.) on this point showed, for example, that beer produced from a wort containing 5.59 per cent. of nitrogenous substances, by means of one and the same type of yeast, contained 4.42 per cent. of these substances when fermented in the cool cellar of the brewery, and 5.10 per cent. when the fermentation was completed in the laboratory (at 66° F.). The characteristic disagreeable flavour produced in beer by fermentation at an unduly high temperature is due to this increased formation of metabolic flavouring products, which will be the more noticeable in proportion as the other (flavouring) extract constituents is smaller. For this reason beers of this class (especially Pilsen) must be fermented at a lower temperature than those of the Bavarian type.

Finally, it should be mentioned that the fact of the migration of nitrogenous substances from the yeast to the nutrient medium renders illusory the results of all investigations in which it is sought to ascertain the quantity of nitrogenous matter assimilated by yeast from the difference in the nitrogen content of the medium at the beginning and end of the experiment. Consequently this question also needs reinvestigation by the application of more delicate methods of experiment.

CHAPTER LI.

CULTIVATION AND REPRODUCTION OF YEAST.

§ 264.—Hansen's Method of Single-Cell Culture.

In samples of yeast as they reach the laboratory from natural sources or from the fermentation industries, the cells are not infrequently in an enfeebled condition, and therefore need to be reinvigorated before they can be sowed in the nutrient gelatin employed for making pure cultures. Thus in the case of breweries, for instance, the usual practice adopted for sending yeast samples to a pure-culture laboratory through the post is to place a drop of the thick balm, about the size of a pea at the most, on a sterilised filter-paper, and thus free it from water to a sufficient extent to enable it to be sent, enclosed in several layers of the paper, in an envelope. On reaching the laboratory this desiccated drop is placed in wort, where the cells are revived and regain their full power. The same thing is done in the case of wine lees. The reinvigorated sample is then subdivided, in the manner described below, in order to obtain cultures that are indubitably grown from a single cell, and are therefore termed "single-cell cultures."

The insecurity of the dilution method has already been pointed out in vol i. (p. 97); and for the purpose of obtaining pure yeast cultures, this method was improved upon by E. Chr. Hansen (II.) in 1879. Hansen had observed that when several cells were present in a culture vessel they settled down separately, when properly stirred, and being devoid of locomotive power, each developed into a colony by itself. In such cases those in which only a single colony developed were alone suitable for the purposes of pure culture. The first six species of Saccharomycetes introduced into the literature by Hansen, namely, *S. cerevisiæ I.*, *S. Pastorianus I.–III.*, *S. ellipsoideus I.* and *II.*, were obtained in this manner. At a later date (1883) Hansen (XII.) made use of the liquefiable solid medium, 10 per cent. wort-gelatin. J. Chr. Holm (IV.), in a critical investigation of the resulting priority controversy, established the fact that this was done by Hansen independently, and especially so with respect to the method of R. Koch.

As already stated in vol. i. (p. 102), it is only capable of furnishing true pure cultures when the experimenter has succeeded, by shaking, in distributing the sample sown in the nutrient gelatin so uniformly that the cells are all embedded separately in the solidified gelatin stratum. Despite the opinion of G. Topf (I.) to the contrary, this condition is not always attainable. By means of an artificial mixture of beer yeast with *Sacch. apiculatus*—which is recognisable from the peculiar shape of its cells—Hansen (XII.) showed that about 2 per cent. of the colonies on the resultant plate cultures were impure, *i.e.*, contained cells of both species. In a special experiment with a series of pure yeast, partly alone and partly in artificially prepared mixtures, J. C. Holm (IV.) showed that from 108 to 135 cells formed the basis of 100 colonies obtained on nutrient gelatin plates by the Koch method. Still more unfavourable are the conditions in cases occurring in laboratory practice, where natural mixtures have to be separated in which the mutual connection of different species is, for various

reasons, of more frequent occurrence and more intimate. Thus P. Miquel (IX.) found, in a case of air analysis, that out of 442 colonies—of which 385 consisted of bacteria—only 136 contained a single species, whilst 87 contained two, 75 three, and 87 four or more species, and Lafar (I.) has shown that mixed colonies, formed of yeast and bacteria together, also occur. Hence, in view of the small dimensions of the cells, the only way to remedy the unreliability of the plate-culture method in the case of bacteria is by making repeated cultures, as recommended in vol. i. chapter xi. With yeast, on the other hand, it is much easier to overcome the defects of the method and obtain cultures undoubtedly arising from a single cell, the cells being large enough (usually 5–10 μ) to enable a low-power objective to be used, the longer focus obviating the risk of contact with the gelatin layer under examination. Consequently the freshly moulded plate, inoculated with the sample to be subdivided, can be examined with a low power (40–60) for the purpose of discovering cells that, in addition to being alone, are far enough from their neighbours to ensure the isolation of the resulting colonies and enable reinoculations to be made from these without incurring the risk of including members from other colonies in the transfer. The position of these suitable cells in the gelatin stratum is noted down at once, so that they may be readily identified later and used for cultures that shall be indisputably descended from a single cell and therefore pure cultures in the strictest sense of the term.

At present we can only deal briefly with the technique of the preparation of single-cell cultures, the reader being referred for more complete details and modifications of the method to the special works on the subject, notably the instructions given by E. C. Hansen (XV. and XXXV.) himself, and A. Klöcker's book (VII.) detailing the experience gained in this branch at the Carlsberg laboratory. A small portion of the sample (previously reinvigorated, if necessary) is placed in sterilised water—or preferably in a 0.5 per cent. solution of common salt—and shaken up so as to separate the agglomerations of yeast and distribute the cells as uniformly as possible. The number of cells in a single drop of the diluted mass having been examined under the microscope, sufficient is transferred to a flask, already charged with 20–100 c.c. of liquefied wort-gelatin (or must-gelatin) to ensure that a tiny drop of this latter contains only a very few cells. To prepare a miniature plate culture, a large cover-glass is sterilised, by passing it through a spirit flame, and one side of the same is then coated with a layer (about 0.2 mm. thick) of the inoculated gelatin, by means of a small platinum loop, in such a manner as to leave an outer ring of clear glass several millimetres wide. The cover-glass is then laid on the vaseline-coated ring (*c*) of a sterilised Böttcher cell (Fig. 159), the bottom of which has already been coated with a minute quantity of sterilised water (*d*). If this moist cell be laid horizontally on a suitable foundation and exposed to a medium room temperature (15° C.), the gelatin film (*b*) on the inside will quickly set evenly. A hollow-ground microscope slide may also be used in place of the Böttcher cell. It is advisable to make several of these plate cultures and not merely a single one. As soon as the gelatin film has set, the cell is examined under the microscope, preferably one fitted with a nose-piece to enable the two requisite objectives of different power (*e.g.*, Zeiss Nos. A and D) to be rapidly exchanged. The low power (about 40–60) is used for systematically examining the gelatin film all over, to find suitable cells that are isolated and far enough away from all others. The higher power (about

Fig. 159.

Böttcher Cell in vertical section. Slightly reduced. (*After Hansen.*)

250–400) is then used to make sure, and the exact position of the selected cells is noted down. The various methods and appliances for fixing the position of the cells in single-cell culture have been critically reviewed by H. Will (XXIV.) on the basis of his wide experience. The author finds the simplest and most convenient method—and much less troublesome to the beginner than the object-marker—is that of marking the position of the cell direct on the glass (the low power being used so that the objective is about 1 cm. away from the slide) by means of two fine dots of ink or preferably black varnish, applied to the top surface of the cover-glass by means of a fine drawing-pen or a pointed inoculating hook. About half a dozen or more cells are marked off in this way, a necessary precaution, because it often happens that one or more of those selected remains barren, either because it was already dead at the commencement of the test or else was too feeble to develop in the strong (10 per cent.) gelatin medium. The task of seeking out the cells is troublesome, even for the experienced worker.

Fig. 160.
Chamberland Flask.
About one-fifth natural size.
(*After Hansen.*)

Fig. 161.—Pasteur Flask, with Hansen's improved modification by widening the swan-neck tube. About one-fifth natural size. (*After Hansen.*)

It may be greatly lightened by using cover-glasses cross etched in squares of about 2 mm. side, as recommended by Will, and preferably marked with etched figures as advised by Alfred Jörgensen. This is also desirable on account of the ease with which it enables the position of the cells to be entered in the note-book, and facilitates keeping a record of the observations. When the marking is finished, the Böttcher cells are placed in a large double basin and left for about twenty hours in the thermostat at about 20° C., by the end of which time the cells that are capable of development will be actively forming colonies. After another twenty-four hours the colonies will be visible to the unaided eye, as small white dots. The selected colonies are again examined under the low power, for the presence of dangerous neighbours, and inoculations are made from each colony into flasks already charged with sterilised nutrient medium (wort, wine, must, &c.), another examination being afterwards made to make sure that the selected colonies alone have been drawn upon and that the adjacent ones are intact. When an inoculating hook is used, it will be found more convenient to transfer the cells to a Freudenreich or Chamberland flask (Fig. 160), and then, after further reproduction, inoculate from this with a loop into a Pasteur flask (Fig. 161), with a capacity of about 250 c.c. and charged with about 100 c.c. of wort. On the other hand, the inoculation can be conveniently effected into the latter culture vessel direct when use is made of a

platinum wire, about 1 cm. long and 0.5 mm. thick, held with a forceps and sterilised in the flame just before using.

Another result—either subsidiary or as the main object—obtainable by means of the above method of reinvigoration, is a purification of the sample. In this way Müller-Thurgau (XX.), for instance, in cultivating yeast destined for the fermentation of red wine or perry musts (rich in tannin) and therefore required to be able to stand the presence of large quantities of that substance, allowed the sediment forming the raw material to reproduce several times in red must or tart perry must. In this way he effected a selection affording greater prospects of ultimate success in the cultures afterwards prepared by the single-cell method.

P. Lindner (XXI. and XV.) applied the name "small drop" culture to a modification of Hansen's single-cell method. He diluted the isolated sample to such an extent with wort that a small drop of same contained only a single cell, and then, by means of a very fine sterilised drawing-pen, made a number of small drops or fine streaks on a sterilised cover-glass, which was next fixed over a Böttcher cell or hollowed slide, by means of vaseline, &c., and examined under the microscope, each small drop found to contain only one cell being marked. The resulting colonies are afterwards transferred to another medium (wort-gelatin, wort). The shrinkage which takes place in cells placed in the highly plasmolytic nutrient gelatin is here avoided, and consequently the proportion of cells that will not develop is reduced. Moreover the cells are more readily detected than is the case with solid media, owing to the greater difference between the refractive power of the cells on the one hand and the medium on the other. F. Schönfeld (II.) found it useful to combine the externals of this method with the essential features of the Hansen single-culture method, by treating a little of the sample (sludge or sedimental yeast) with wort-gelatin and transferring small drops and streaks of this mixture to a cover-glass which is afterwards fastened down on a hollowed slide.

Reinoculation from a single-cell culture into a fresh nutrient medium gives an absolutely pure culture. The labour devoted to the latter has then a different purpose, more especially to determine whether it is adapted to the object in view, to select the most suitable from a series of pure cultures, and finally to reproduce a sufficient quantity for use in practice.

A few remarks may be made, for the information of beginners, on the best method of keeping pure cultures in the laboratory. The most convenient, when feasible, is to leave the yeast covered by the liquid in which it has been grown. This is only practicable, however, when the operator has sufficient leisure or inducement (in the way of orders for supplies of yeast) to re-transfer the cultures to fresh medium at frequent intervals. If this be neglected, and the cells are left for a long time in the spent liquid, they will be injured by the products of metabolism and fermentation. The length of the intervals within which this reinoculation must be performed, in order to prevent the danger aforesaid, depends on the species of yeast as well as on the constitution of the nutrient medium. Thus, H. Müller-Thurgau (III.) states that he was able to leave certain wine yeasts in their respective wines for ten months without appreciable injury. On the other hand, the author found that a spirit yeast, Race II., suffered a transitory, but decided, weakening (especially in reproductive power) by remaining only three months in the fermented wort. The live herbarium in the laboratory of a fermentation physiologist, that is to say, the collection of cultures of micro-organisms, usually contains a very large number of yeasts. These include certain kinds—especially yeasts for the production of wines and berry wines, then the so-called wild yeast, and others—for whose reinoculation there is little or no outside demand. Nevertheless the reinoculations take a

good deal of time and labour when—as is desirable *per se*—the collection is a large one numerically. In order to reduce the work to a minimum the yeast should be transferred to the medium in which it is known to keep longest without suffering a change of character. Beer wort is not suitable for the prolonged storage of brewery yeasts; but, on the other hand, the aqueous 10 per cent. solution of saccharose tried and recommended by E. C. Hansen (XXXV.) usually behaves well. It is generally kept in Freudenreich flasks or in the Hansen modification shown in Fig. 162, these being filled about half full. To minimise the inevitable evaporation, J. C. Holm (III.) employs a modified form of cap or hood. The amount of sediment of the culture to be preserved must not be more than imparts a slight turbidity to the liquid, the reproduction of the sowing and the chemical alteration of the liquid being then very slight. The object in view is not the reproduction, but the preservation of the cells, and it is therefore necessary to prevent as far as possible the distribution of the store of nitrogenous food introduced by the cells and consumed for their own maintenance during storage, or a weakened progeny will certainly result. Moreover, the reinoculations should be kept in the dark and at a temperature not far exceeding 15° C. Under these conditions nearly all the species (about 50 in number) examined in this connection by Hansen (XXIV., V.) retained their vitality for many years (up to seventeen). *Sacch. Ludwigii* proved less hardy, some of the samples dying within one-two years, though others lasted six years; and Carlsberg bottom yeast No. 2 behaved in a similar manner. To these exceptions must be added the *Sacch. theobromæ* isolated by A. Preyer (I.) from putrefying cocoa, and also—according to J. C. Holm (II.)—a few species of *Schizosaccharomyces*, one of which perished within a year in saccharose solution, though found to be still living in wort after two and a half years. Beer wort, on the other hand, proved unreliable with all the others tested for this purpose. True, in not a few instances, living cells were found in it after ten or even eleven years, but just as frequently none could be detected at the end of a single year. This liquid is subject to considerable variations in constitution which cannot be influenced or ascertained by the experimenter. In countries where the summer is hot, provision must be made at that season for artificially cooling the cultures (in an ice-chest of special construction); otherwise, as was observed by H. Will (XIX.) in Munich, and by A. Kukla (I.) in Prague, degeneration and death will occur even with saccharose solution. In consequence of unfavourable experiences, A. Jörgensen (IX.) recommends that streak cultures on wort-gelatin should not be employed for storage purposes. The tendency to and results of the formation of films on ageing cultures have been already referred to on p. 87, vol. ii.

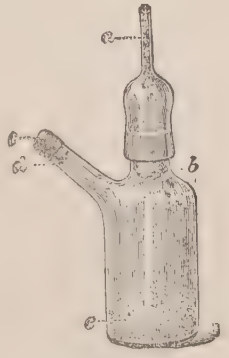


Fig. 162.
Freudenreich-Hansen Flask. About half natural size. (*After Hansen.*)

The colour of beer wort is ameliorated by the fermenting yeast, not solely (as is known to every brewer) during primary fermentation, but also, and to a far greater extent, when the fermented beer is left for a long time in contact with the sedimental yeast, as is the case when yeast cultures are stored in wort. A gradual and extensive decoloration of the nutrient medium occurs (*see* p. 92, vol. ii.); so much so, in fact, that the colour may be changed from brown to pale straw-yellow. The experiments conducted on this point by H. Will (XXV. and XXII.) showed that the wild yeasts generally are more powerful decolorants than the culture yeasts.

When a pure culture has to be transferred from one laboratory to another,

which possesses the necessary equipment for further reproduction, a small quantity of the culture—even a couple of vigorous cells will suffice—is placed on a little cotton-wool and inserted in a previously sterilised Freudenreich or Freudenreich-Hansen flask. The cotton should not have been freed from fat, and will then be hygroscopic enough to supply the yeast with the requisite minimum of moisture to prolong its vitality. The Hansen modification of the Freudenreich flask (Fig. 162) differs from the original type by the provision of a slanting tube at the side to facilitate connection with a Pasteur flask, both for introducing a drop of the transferred culture on to the cotton (*e*) and also for inoculating it into a fresh nutrient solution. A coating of sealing-wax (*c*) makes a tight joint over the stopper of rolled asbestos-paper (*d*). The plug of cotton (*b*) in the neck of the flask prevents over-rapid and excessive desiccation of the contained cells, and at the same time excludes any extraneous germs that may have slipped through the plug (*a*) in the cap tube. This method of storage on cotton is of special value, and in fact indispensable, when perfectly pure cultures are to be sent to tropical countries and will be exposed to high air temperatures for some time *en route*. Streak cultures on gelatin or agar-agar, which are occasionally used for transport over short distances in the temperate zone, are out of the question in such cases.

§ 265.—Conditions of Cell Reproduction.

Of the two possible methods of cell reproduction in yeast, namely, budding (the vegetable form) and ascosporulation, the second may be disregarded so far as the practice of yeast culture is concerned, on account of the greatly restricted increase it furnishes. The method of budding, with which alone we shall now have to deal, has already been treated of in § 245, though only from the standpoint of the morphologist, the chief point being the variability in the form and aggregation of the cells resulting from vegetative reproduction. In the present paragraph this information will be supplemented by a review of the external conditions of cell reproduction, the requisite constitution of the nutrient medium having been already dealt with in the two previous chapters. Before, however, entering upon a discussion of this question, it will be necessary to treat of the limitation of three definitions which here come into application.

The term "reproductive capacity" (occasionally also known as "coefficient of reproduction") relates to the total number of cells in the yeast crop obtainable under any given conditions (*i.e.*, independently of time) per unit of the original sowing. This unit may be taken on the basis of weight, in which event a reproductive capacity of 20, for instance, implies that 20 grms. of yeast crop have been obtained for every 1 grm. of yeast sown. The crop is usually obtained in the form of a sediment (*see* vol. ii. p. 82), and is weighed either in the pressed condition (with a variable content of water according to the degree of pressure) or else after drying. The latter method is the most reliable, especially in experiments on the consumption of nutrient substances, provided—as we know (vol. ii. p. 131) is not usually the case—the deposit consists of yeast cells exclusively. For this reason statements, based on unit weight, relating to the ratio of increase are of merely low and conditional value. Far greater comparative reliability attaches to determinations made with the cell-counter (vol. i. p. 97), and referred to the unit of cells in the sowing.

"Reproductive power" (also spoken of as "the velocity of reproduction" or "reproductive energy") indicates the number of cells produced from the unit of sowing in unit time. With regard to the determination of this value, the remarks just made also apply.

By the "period of generation" is generally understood the interval of time

necessary for the production of a fully developed daughter cell from the parent cell by budding. If F. Baseneau's formula (I.) (vol. i. p.45,) is used for this determination, instead of the method of direct observation of the individual cell, it must be remembered that this formula does not strictly apply, except under the condition that the duration of generation has been the same for all cells in the culture throughout the whole experiment, and that each newly formed daughter cell has immediately begun to act as a parent cell. Since this latter condition in particular is not usually fulfilled in its entirety, the value furnished by the calculation is higher than the truth.

The influence of temperature on cell reproduction by budding deserves very close consideration, because the use in practice of nutrient media which are mostly of almost constant and unchangeable constitution, renders it possible to control the progress of reproduction by graduating the influence in question. The limits of temperature within which cells will bud in beer wort have been determined by E. C. Hansen (XXXII.) for eleven different *Saccharomycetes*. The upper limits were: for *Sacch. Past. I.*, 34° C.; for *Sacch. membranæfaciens*, *S. Ludwigii*, and Wortmann's wine yeast, Johannisberg II., 37° C.; for *S. cerevisiæ I.*, *S. Past. II.* and *S. ellips. II.*, 40° C.; for *S. ellips. I.*, 40–41° C.; and for *S. Marxianus*, 46–47° C.; whilst the lower limits were as follow: *S. cerevisiæ I.* and *S. membranæfaciens*, 3° to 1° C., *S. anomalus*, 1° C. to 0.5° C., and for the other eight species 0.5° C. A comparison of these figures with those in the table opposite p. 98, vol. ii., will confirm the remark on pp. 94, 95, that the limits of temperature are somewhat wider in the case of budding than for sporulation, provided the former occurs in wort. If, on the other hand, the cells be allowed to bud in water, the higher limit of temperature is slightly reduced, whilst the minimum is raised. *Saccharomycopsis guttulatus* will only bud at 37° C.

The influence of temperature on the period of generation was first closely investigated by Rasmus Pedersen (II.), after a few observations on the subject had been communicated by Pasteur and others. Working with a low-fermentation beer yeast in unhopped wort (16.2° Balling), Pedersen found that the duration of generation in the first 24 hours of development was 20 hours at 4° C., 10½ hours at 13.5° C., 6½ hours at 23° C., 5.8 hours at 28° C., and 9 hours at 34° C., no budding occurring at 38° C. Consequently the optimum temperature giving the shortest period of generation in these experiments was between 28° and 34° C. At a later date a more comprehensive test was performed by D. P. Hoyer (I.) by fixing a small number of cells, in a state of watery suspension, on a thin film of solidified wort-gelatin applied to the under side of the cover-glass of a Böttcher cell. The preparations, which could be examined and counted direct, were maintained at the desired temperature for a certain time, the cells produced in the interval being then counted and the period of generation calculated by means of the formula already mentioned. The following results were obtained at 13° C.: *Sacch. Pastorianus I.*, Hansen, 6 h. 6 min.; *S. Past. II.*, 8 h. 45 min.; *S. Past. III.*, 8 h. 39 min.; *S. ellipsoideus I.*, 9 h. 4 min.; *S. ellips. II.*, 8 h. 49 min.; *S. anomalus*, 5 h. 12 min.; *S. Ludwigii*, 8 h. 10 min.; *S. membranæfaciens*, 7 h. 1 min.; Saaz yeast, 7 h. 48 min.; Frohberg yeast, 7 h. 21 min.; *Sacch. apiculatus*, 4 h. 45 min., &c. The period of generation at 25° C. was, for *S. Past. II.*, 5 h. 12 min.; *S. Past. III.*, 6 h. 8 min.; *S. ellips. I.*, 6 h. 12 min.; *S. ellips. II.*, 6 h. 9 min.; *S. membranæfaciens*, 5 h. 13 min.; Saaz yeast, 4 h. 23 min.; Frohberg yeast, 4 h. 18 min. In the same proportion as new cells are formed, the medium is impoverished of the necessary structural materials and enriched with metabolic products inimical to development. Both these influences grow quicker when the nutrient medium is kept warm, and then soon become so powerful that the velocity of reproduction falls

below the value previously obtained from cultures that have been kept much cooler, while at the same time the period of generation is correspondingly increased. Thus in Pedersen's experiments, cited above, the period of generation on the second day, though 20 hours at 4° C. and 16.7 hours at 13.5° C. was increased to 65 5 hours at 23° C. Further details will be given on this point in that portion of the next chapter which deals with the influence of alcohol on the life of yeast. In the case of temperatures near the limits beyond which budding ceases, the velocity of reproduction is so low as to be regarded as nil from the practical standpoint, H. Müller-Thurgau (XIX.) having reported, for instance, that the wine yeasts examined by him ceased to reproduce at 40° C. In climates where the temperature at the time of the vintage approaches this limit —the South of France, for example, according to Kayser and Barba (I.)—the must has to be artificially cooled, or the yeast cells present will reproduce so slowly that only a sluggish fermentation will be set up, incapable of suppressing concomitant injurious organisms. In northern vineyards, on the other hand, the autumn temperature not infrequently approaches the lower limit for yeast reproduction; and in that event the fermentation of the must is greatly retarded and slow.

In breweries using low-fermentation yeast the wort is pitched at 5°–7.5° C., and during primary fermentation the temperature is not allowed to exceed 9° C. for beer of the Bohemian type, 9.5° C. for Vienna beer, or 10.5° C. for Bavarian beer; so that the temperature throughout remains considerably below the optimum reproduction temperature. The usual amount of pitching yeast, namely, about one part by volume of thick barm per 2000 of wort, causes fermentation to start quickly; but when, from any cause, the brewer is restricted to the use of a smaller proportion of yeast, he does not allow this to act on the whole mash at once, but first puts it through a reproduction process in a portion of the wort that has been cooled to 15°–20° C., and is maintained at that temperature. Reproduction ensues rapidly and abundantly, so that in a few hours the culture, which is beginning to throw up a "head," can be used for pitching the rest of the wort at the usual temperature.

Particulars on the influence of temperature on the reproductive capacity of yeast have also been published by A. L. Stern (III.), who, cultivating Burton yeast in a mineral salt nutrient solution treated with dextrose and asparagin, obtained the maximum weight of yeast crop at a temperature between 21° and 25° C.

The influence of the constitution of the nutrient medium on the reproductive capacity has been established by a series of observations. With regard to the source of nitrogen, F. Hess (I.) was able to prove the superiority of yeast water over mineral-salt solutions containing sufficient asparagin or peptone to furnish an approximately equivalent quantity of nitrogen (9.0, 8.2, and 8.5 mgrms. per 100 c.c.) and sugar, the following results having been obtained in each 100 c.c. of the liquid during 28 days, the number of cells being referred to each cell in the original sowing (20 cells per c.c.):

Source of Nitrogen.	Reproductive Capacity of		
	Saaz.	Frohberg.	Logos yeast.
Asparagin	536	601	645
Peptone	704	1580	2037
Yeast water	1529	1659	3608

A similar result was obtained by C. Soldan (I.); and the reports of P. Thomas (I.) and A. L. Stern (III.) have already been mentioned in vol. ii. on p. 156. A certain influence is also exerted by the nature of the source of carbon, as has been shown by Soldan, the Saaz, Frohberg and Logos yeasts exhibiting the highest reproductive capacity when maltose was added, as source of carbon, to nutrient media consisting of mineral-salt solutions containing asparagin or yeast water. Dextrose furnished a medium crop, and saccharose gave the lowest.

The influence of the concentration of the medium on the reproductive capacity is stated by R. Pedersen (II.) to be small. More exhaustive experiments were instituted in 1887 by J. Archleb (I.), pressed yeast (1 grm. per litre) being treated with malt extract ranging in strength from 1 to 25 per cent., the largest yeast crop was furnished by the 14 per cent. solution. In the case of a Burton yeast grown by A. J. Brown (VII.) at 20° C. in hopped ale worts of various strengths, it gave no increase in crop when the concentration exceeded about 15 per cent. Balling. A. L. Stern (III.), who grew Burton yeast in two sets of experiments in which the asparagin content (3 grms. and 1.5 grms. respectively) was constant, whilst the dextrose varied between 0 and 30 per cent., obtained the maximum yeast crop with 15 per cent. of sugar in the one case and 12.5–15 per cent. in the other. Again, the experiments of Emil Bauer (III.) show that any excess of nitrogenous nutriment (whether in the form of yeast extract or of the autodigestion products of yeast) beyond the amount absolutely necessary has no influence on the crop.

Nutrient solutions which are very rich in sugar, and therefore have a strongly plasmolytic action on the cells, retard or even entirely prevent reproduction, a circumstance that is utilised in cookery for the preservation of fruit. No generally applicable data can be given respecting the proportion of sugar necessary to cause plasmolysis, because the corresponding influence of other ingredients in the solution also comes into play. In the experiments of E. Laurent (VI). with a number of beer and wine yeasts, the appreciable reproduction ceased in the cultures in which the nutrient solution (a decoction of malt culms) contained about 60 grms. of saccharose, invert sugar, dextrose or maltose per 100 c.c. Greater powers of resistance in this respect are possessed by *Sacch. Zopfii;* and the yeast isolated by E. Dubourg (II.) from sweet Sauterne proved capable of acting in an 80 per cent. solution of invert sugar. A. Mayer (VI.) claimed that the plasmolytic action of a 30 per cent. sugar solution could be counteracted by a small percentage of Seignette salt, the addition of which soon caused abundant reproduction and powerful fermentation in a previously quiescent nutrient medium; but this was disproved by M. Hayduck and M. Delbrück (I.). Greater powers of withstanding the influence of high percentages of sugar and other ingredients in the nutrient medium are possessed by the yeasts concerned in the (spontaneous) fermentation of Danzig Jopen beer (chiefly exported to England, under the name spruce beer or black beer). This was demonstrated by P. Lindner (XIV.), who isolated two new species of yeast, *Sacch. farinosus* and *Sacch. Bailii*, from fermenting Jopen wort of the initial concentration 53–54 per cent. Balling.

The relative permeability of the cell membrane also affects the reproductive power. This permeability varies, not merely in the different races of yeast, but among individuals of the same race, and is determined by numerous factors, more particularly the age of the cells and the conditions of its earlier life. Hence both the fecundity and reproductive power differ, under identical external conditions, with the species (or race) of the yeast. On this point P. Lindner (XX. and XIV.) in 1889 instituted comparative experiments with twenty-two beer yeasts and fifteen white beer, spirit and pressed yeasts. The former, sown in

650 c.c. of hopped wort (11.95° Balling), furnished from 4.3 to 12 grms. of crop, weighed after pressing, whilst the yield from the second group, sown in 1350 c.c. of wort, was 9.3 to 19.5 grms. The highest yield was obtained from the white-beer yeasts, the pressed yeasts coming second. Further contributions on this matter have been published by F. Schönfeld (I.), C. Soldan (I.), F. Hess (I.), Roderich Meissner (I.), G. Korff (I.), W. Knecht (I.), and others.

The influence of the age of the sowing on the reproductive capacity and reproductive power was examined by M. Elliesen (I.) in the case of Frohberg and Logos yeasts. Some of his results are given below:

Age of Cells in the Sowing.	Reproductive Capacity in 42 days at 6°–8° C.		Reproductive Power in 8 days at 6°–8° C.	
	Frohberg.	Logos.	Frohberg.	Logos.
24 hours	2880	6400	144	320
3 weeks	800	3200	40	160
8 weeks	1200	720	60	36

With increasing age the vitality and reproductive power of the cells are weakened. Whilst the gradual thickening of the cell membrane is a good defence against adverse influences on the part of a nutrient medium that is changed for the worse, it equally presents obstacles which must be overcome by the stimulating ingredients of a fresh medium before the sowing can reproduce itself and lay up new material. The far higher reproductive capacity of the day-old sowing in the above table is therefore not surprising.

An important point in fermentation technology is the influence of the extent of the sowing on the amount of crop that can be produced in a nutrient medium of given quantity and composition. This applies to the brewer and vintner, as well as to the yeast manufacturer. The first reliable determinations obtained under practical working conditions were those of Thausing (I.), who instituted parallel experiments in four Austrian breweries (A to D), the wort being pitched with 0.33, 0.5 and 0.66 litre of thick low-fermentation barm per hectolitre respectively. The results are expressed in the following table, which shows, in the first place, the total crop from each sowing, and then the actual increase (crop *minus* sowing):

Sowing of thick Barm per Hectolitre of Wort. Litre.	Resulting Crop of thick Barm. Litres.				Increase—thick Barm. Litres.			
	A	B	C	D	A	B	C	D
0.33	1.63	1.99	1.74	1.49	1.30	1.66	1.41	1.16
0.50	1.61	1.95	1.95	1.50	1.10	1.45	1.45	1.00
0.66	1.66	1.83	1.79	1.53	1.00	1.21	1.13	0.87

It is thus evident that while an increase in the amount of the sowing has little effect on the total crop, it causes an appreciable diminution of the increase. This fact has been repeatedly confirmed: for instance, by O. Reinke (II.) in 1889 for low-fermentation beer yeast; by A. J. Brown (VII. and IV.) in 1890

and 1892 for Burton top-fermentation yeast; in 1897 by Thausing himself in new experiments; and in the same year by A. Reichard and A. Riehl (II.) at the low-fermentation brewery at Lutterbach (Elsass), and by the Bierbrouwery d'Oranjeboom (I.) at Rotterdam.

The latter also confirmed Thausing's discovery that the accuracy of the results is unaffected by the height of the temperature, it being immaterial whether the fermentation was conducted at a low temperature (starting at 3.8° C. and rising to 9.4° C., to afterwards recede to 6° C.), or in the warm, commencing at 10.1° C., rising to above 15.8° C., and afterwards declining to 10° C., or finally carried on at the usual level of 8° C., 8.8° C., and 11° C. In low-fermentation breweries it is customary to pitch the wort with $\frac{1}{2}$ per cent. by volume of thick barm, which increases about eight-fold during fermentation. The resulting deposit of yeast at the bottom of the fermentation vessel consists of three layers: bottom, middle or core, and top, the middle one being carefully separated from the other two and alone used for pitching subsequent brews. It forms about 60 per cent. of the total deposit, but suffers diminution during the washing process to which it is subjected, the final yield being only about twice the original amount of the pitching yeast.

§ 266. Consumption of Oxygen for Cell Reproduction and Respiration.

The question whether strictly anaerobic species of yeast (*see* vol. i. p. 139) exist, and therefore whether cell reproduction can proceed, without restriction, in the entire absence of free oxygen, cannot yet be finally settled. J. Behrens (VIII.) reports that he found a comparatively large number of anaerobic yeasts on hop cones, and Traube (II.) had previously stated that yeast reproduction may proceed, in certain circumstances, in the absence of oxygen. On the other hand, Brefeld (XIII.) considered he had proved the absolute necessity for the presence of oxygen. G. Korff (I.) observed a considerable degree of reproduction in cultures in yeast water treated with 10 per cent. of saccharose and traversed uninterruptedly by a current of hydrogen, the yeast sowing employed having been grown under similar conditions. At the end of fourteen days the amount of crop obtained per original cell was: Saaz yeast, 876 cells; Frohberg yeast, 1346 cells; and Logos yeast, 1160 cells. According to P. Barker (I.) a *Saccharomyces* isolated from ginger exhibited no signs of incipient growth when oxygen was completely excluded.

The more closely we criticise the reliability of the methods and appliances hitherto used for obtaining really anaerobic conditions in cultures, the smaller the trust we place in the results of the experiments made in this connection, and the greater becomes the doubt whether this or that anaerobic is one in the strict sense of the term or merely an aerobe requiring only a very small quantity of oxygen. We need only remember how difficult it is to completely eliminate oxygen from the gas (hydrogen, nitrogen, or carbon dioxide) employed to displace the air and traverse the cultures; and the non-fulfilment of this preliminary condition will certainly influence the results when we are dealing with organisms that are very sensitive toward oxygen and are stimulated by very small quantities of that gas. Now yeast is an organism of this kind, being, as Brefeld (XIII.) observed, satisfied with a tension corresponding to the presence of 1 part of oxygen in 6000 parts of carbon dioxide. Traube's counterproof with the assistance of sulphindigotic acid cannot be accepted unconditionally; and the fact that yeasts will reproduce during the temporary exclusion of oxygen is insufficient as proof, since in such cases the yeasts consume the previously accu-

mulated store of that element. Moreover, cultures in which the vessel is provided with a seal of dilute sulphuric acid during fermentation cannot be regarded as having developed in the absence of oxygen. E. C. Hansen (XXXII.) states briefly that budding occurred in his experiments with nitrogen freed from oxygen; but there is a decided difference between this and sporulation, which takes place only in presence of an abundant supply of oxygen.

As a matter of fact, the growth and reproduction of all the yeasts hitherto examined proceed more freely in aerated cultures. The first results that can be classed as reliable (because obtained with the counting cell) were obtained in 1879 by E. C. Hansen (XXX.), who showed that a certain beer yeast when grown in wort at 12°–14° C. increased 11.2-fold in 60 hours without aeration, and 15.8-fold with admission of air. In a second experiment, at 13°–15° C., the crop was 9 and 27.3 cells respectively per original single cell. Other workers afterwards obtained similar results. The assertion of N. von Chudiakow (I.) that oxygen is indispensable for yeast reproduction only in the case of imperfectly nutritive media, does not seem to rest on a sufficient foundation. Other conditions being equal, the degree of stimulation imparted to cell reproduction by aeration depends on the race of the yeast—a fact observed by G. Korff (I.), M. Delbrück (I.) and several other workers. Nevertheless, as Prior (II.) has shown, the greater fecundity observed in aerated cultures is attributable not merely to the excess of available free oxygen, but also to the circumstance—which should not be under-estimated—that the gas traversing the nutrient medium frees the latter from various products (volatile acids) of yeast metabolism that retard reproduction.

Yeast has the power of absorbing free oxygen from the environment, utilising it in completing internal chemical changes, and then excreting the most part in the form of carbon dioxide. The closer examination of this process of respiration is illuminative. The quantity of oxygen taken up by the yeast cell has been determined by P. Schützenberger and E. Quinquaud (I.), in an experiment wherein pressed yeast (containing 26 per cent. of dry matter) was distributed in aerated water, the figures per 1 grm. yeast in 1 hour being 0.1 c.c. at 9° C., 0.4 c.c. at 11° C., 1.2 c.c. at 22° C., 2.1 c.c. at 33° C., 2.1 c.c. at 40° C., 2.4 c.c. at 50° C., and 0.0 c.c. at 60° C. These workers assert that no further absorption of oxygen takes place in media which, like arterial blood, are capable of ceding 200–230 c.c. of oxygen per litre, instead of the 6–7 c.c. present in the water used, but this is true only when the experiment is conducted under the conditions employed by them. By working under different conditions, A. Harden and S. Rowland (I.) found that 1 grm. of pressed yeast can take up an average of 3.54 c.c. of oxygen per hour. Moreover, the consumption of oxygen would probably reach a high figure if the cells were abundantly supplied with respirable substances, instead of, as in this case, being compelled to feed on one another. In fact, it has been found by Giltay and Aberson (I.) that yeast grown in a medium containing sugar consumed more and more of that substance in proportion as the degree of aeration was increased and the mixture of air and oxygen was richer in the latter constituent, as much as 21 per cent. of the total sugar (utilised for cell construction, respiration, and fermentation) being consumed in this way. The higher final attenuation of wort that has been strongly aerated during fermentation is partly due to the increased respiration.

Few of the investigations made in connection with the dependence of yeast respiration on external conditions are reliable. Since the discovery that alcoholic fermentation by yeast is a purely enzymatic action (*see* chapter lxiii.), and not directly and inseparably connected with the life of the cell, it has become necessary to bear in mind the two-fold character of the sources from which the liberation of carbon dioxide may proceed, and therefore to consider respiration

separately from fermentation. Strictly speaking, the only way in which the influencing of respiration can be reliably tested is by employing conditions in which the nature of the yeast and medium precludes the possibility of any alcoholic fermentation—a stipulation which at once disqualifies a whole series of investigations on the "carbon balance" of the fermentation process, the stimulation of the latter by aeration, &c. Little is known as to the intermediate stages of the combustion of the substances undergoing respiration. *Saccharomyces Hansenii* isolated by W. Zopff (XIII.) from American cotton-seed meal —a yeast incapable of inciting alcoholic fermentation—forms large quantities of oxalic acid as the final oxidation product (instead of carbon dioxide) of the sugars (glucose, galactose, saccharose, maltose, lactose, mannitol, dulcitol, and glycerol) added to the nutrient medium. Several workers, including E. Prior (II.) have found that copious aeration increases the quantity of acids formed in yeast cultures; but it is not yet certain how far these are to be regarded as products of the purely chemical action of oxygen on the constituents of the medium or as the result of hyperstimulation of yeast metabolism. According to the comparative experiments of G. Korff (I.) with Saaz, Frohberg and Logos yeasts, the aerated cultures contain a higher proportion of fixed acids, whilst those traversed by a current of hydrogen furnish a higher yield of volatile acids. In this connection we may recall the remarks made on p. 92, vol. ii., with reference to the divergent chemico-physiological behaviour of the cells of sedimental and film yeasts. The nature of the influence of external conditions on the coefficient of respiration of yeasts (*see* p. 57, vol. ii.) has not yet been sufficiently investigated. Under favourable circumstances the amount of heat liberated by respiration may give rise to a considerable increase in temperature, and in a case observed by Effront (VIII.), in which 2 kilos. of pressed yeast were crumbled down and exposed to the air (20° C.) as a layer 37 cm. in depth, this amounted to 36° C. in three hours.

The utility of aerating the nutrient medium as regards the development of the yeast to be grown therein has long been recognised in practice. During the early stages of rousing (aeration), whilst it is still very hot, the wort absorbs oxygen freely, fixing it by chemical combination and retaining it partly in the form of carbon dioxide. Afterwards, when the temperature has fallen, a considerable amount of the gas is also retained physically (in solution). The quantities were found by Pasteur (III.), in one experiment, to amount to 41.7 and 7 c.c. respectively per litre of wort; but a considerably lower figure (5.7 c.c.) was obtained in the latter case by P. Petit (III.). A. Petersen (II.) detected 2–4 c.c. of oxygen per litre in the worts of the Alt-Carlsberg brewery; and, finally, C. Bleisch and R. Schweitzer (I.), in investigating the connection between oxygen absorption and the temperature, chemical composition, gravity motion of the wort, determined the amount of the physically dissolved oxygen in wort of 14.4 and 14° Balling, as 2.4 c.c. per litre at 62.5° C. and 4.4 c.c. at 5° C., the chemically combined portion, on the other hand, being 53 c.c. at 85° C. and 4 c.c. at 45° C. The decomposing action of oxygen, whilst very mild at medium temperature (15° C.), is nevertheless appreciable when continued for a long time. In this connection reference may be made to the occurrence of formic acid (associated with carbon dioxide) observed by Rayman and Kruis (I.) in old, sterile wort exposed to the air.

The aeration method of yeast manufacture (*see* vol. ii. p. 134) is based on the observation that cell reproduction is stimulated by powerful aeration, and originated in Sweden, though the first incentive was given by H. Hayduck's experiments (V.), subsequently followed up by M. Delbrück (VI.). Under the stimulating influence of oxygen the yield of pressed yeast furnished by this method amounts to 25–30 per cent. of the weight of cereal substances in the

mash, as compared with about 12 per cent. in the old method which is now being gradually superseded.

In the laboratory cultivation of yeast for technical purposes, the aeration of the nutrient medium must not be omitted; for it has been shown by Hansen, and confirmed by A. JÖRGENSEN (X.) that beer yeast grown in imperfectly aerated wort gives unsatisfactory results when used in practice, the "break" of the wort especially being defective.

The influence exerted by oxygen on the progress of alcoholic fermentation will be dealt with in chapter lxiv.

CHAPTER LII.

THE EFFECT OF CERTAIN TECHNICALLY IMPORTANT CHEMICAL INFLUENCES ON YEAST.

§ 267. Copper and its Salts.

In the previous three chapters we have set forth the formal conditions relating to the nutrition and reproduction of yeast; and we have now to deal with certain important adverse influences to which this organism is exposed either in nature or in fermentation on a practical scale. The effect of physical agencies may be disregarded at present, since they are treated of elsewhere, and we confine ourselves for the moment to chemical influences, beginning with those of copper as the first to which the yeast is generally exposed during reproduction or fermentation.

The behaviour of yeast cells toward copper and copper salts is of particular interest to the vintager. As the reader is aware, the ravages of *Peronospora viticola* are combated by sprinkling the vines with "bouillie Bordelaise" (introduced by A. Millardet of Bordeaux), an approximately 3 per cent. solution of copper sulphate in which—as has been shown by Berlese and Sostegni (I.)—the copper is converted into hydroxide (or basic double salt) by the addition of an equivalent amount of calcium hydroxide. Experience has proved that this remedy produces the desired effect, the conidia of the fungus being destroyed, and the leaves and fruit of the vine preserved; but at the same time the copper exerts a toxic influence on the natural yeasts present on the grapes, and since the various races of yeast probably differ in their susceptibility to this action of copper, it may be supposed that the treatment of the vines will result in an alteration in the flora of the grapes, and that its influence will extend as far as the must vat—more especially when sprinkling has been performed late in the season. Thus, A. Rommier (I.) has observed that must from late-sprinkled grapes gave no sign of fermentation, even under favourable conditions of temperature; whilst in other cases the only living cells detected were those of *Saccharomyces apiculatus* (probably less sensitive to copper), so that the fermentation remained incomplete. This observation led Rommier to investigate the influence of copper on yeast cells, with the result that even an addition of 25 mgrms. of Cu (= 98 mgrms. of crystallised copper sulphate) per litre of must was found to retard the commencement of fermentation. On the other hand, P. Pichi (I.) stated in 1891 that the addition of less than 150 mgrms. of copper per litre of must had no adverse influence on the development and fermentative action of the species of wine yeast examined by him. Both workers, however, omitted to bear in mind Polacci's observation that the copper sulphate added to wine must combines with the tartrate present to form potassium sulphate and copper tartrate, which latter, being insoluble, is precipitated and thrown out of action. Observing this precaution, F. Krüger (I.) in 1894 found, in the case of a pure culture Johannisberg yeast, that the maximum amount of copper sulphate—present in solution and therefore active—that the yeast would stand without appreciable injury was equivalent to 44-45 mgrms. of copper per litre. When increased above this limit it gradually diminished the

fermentative power, though the reproductive faculty and fermentative capacity were not so quickly destroyed. In the experiments conducted on low-fermentation beer yeasts by H. Will (I.), a large number of cells, after immersion in a 5 per cent. solution of copper sulphate for twenty-four hours still proved capable of fermenting the sugar solution to which they were transferred.

The assumption that the above figures obtained by Krüger may be of general application was controverted by the important discovery, made by E. Biernacki (I.) in 1891, to the effect that the amount of antiseptic (here copper sulphate) necessary to retard the fermentative activity of yeast varies with the amount of the sowing. This has been confirmed by other workers; for example, by H. Mann (I.), H. Pottevin (I.) and A. Amand (I.) in connection with copper sulphate; by Mann in the case of iron sulphate, lead acetate and corrosive sublimate; by C. Wehmer (XV.) with potassium and sodium arsenite, and by Müller-Thurgau (XVII.) in the case of sulphurous acid. Nevertheless, though the idea of obtaining unconditionally accurate limit values in this way must be abandoned, it is still highly desirable to make further investigations, especially on the comparative sensitiveness of different pure yeasts and more particularly as a contribution to the solution of the important question of the influence of the copper treatment on the modification of the yeast flora of the vine.

It should be mentioned—as might be foreseen from particulars already given in vol. i. p. 91, when copper sulphate is added in far smaller quantities than the above limits, it no longer restricts, but actually stimulates the fermentative activity of yeast. This occurs, according to Biernacki, when the dilution reaches the proportion of 1 part of copper sulphate per 600,000 parts of nutrient solution.

Apart from very exceptional cases, the vintager has no need to fear that any disturbance of vinous fermentation will be caused by the copper sprinkled on the grapes. According to Tschirch (II.), the results of E. Mach's researches—confirmed by the investigations of M. Hoffmann (V.) on Portugese wine—only about one-tenth of the copper on the grapes finds its way into the must, all the rest remaining on the skins. Moreover, it seems from Polacci's researches that only a small fraction of that tenth actually becomes operative, so that but little damage can be caused, and Chuard has shown that this soluble remainder is mostly precipitated as malate and tartrate as the percentage of alcohol increases during fermentation, some portion being also converted into sulphide by the sulphuretted hydrogen (*see* vol. ii. p. 147) produced by the yeast cells. Consequently the clarified wine after separation from the yeast will only contain a few milligrammes of copper per litre, even when made from grapes that have been extensively sprinkled with copper. Of course a correspondingly larger amount will be present in the deposited yeast.

From the researches of H. Mann (I.) and H. Pottevin (I.) it may be concluded that the yeast converts a portion of the copper sulphate in the nutrient solution into copper phosphates ($Cu_2H_2P_2O_8$ and $Cu_3P_2O_8$), whilst another portion is retained by the cells. When a yeast thus enriched with copper is transferred to a colourless nutrient solution, the observations of H. Will (I.) show that the metal (*i.e.*, an unidentified compound of same) passes into the liquid, which acquires a blue tinge. The combination is probably between the metal and substances of the nature of those already referred to as yeast gum on p. 128, vol. ii.; and for this reason also, the figures already quoted above with regard to the toxic action of copper sulphate cannot be regarded as unconditionally accurate.

When pure yeast is grown in copper vessels for use in practical fermentation, the inner side of the vessel in contact with the fermenting liquid must always be well tinned. Otherwise the copper will be corroded, and the yeast crop will contain appreciable quantities of the metal, which is undesirable for several reasons. Thus, H. Seyffert (I.) detected 0.27–0.64 per cent. of CuO in the

ash of pure yeast (*see* vol. ii. p. 144) grown in an apparatus that was defective in this respect. This quantity probably consisted in part of insoluble copper salts formed during fermentation and deposited, and partly of cupriferous constituents of the yeast cells. The tin used for plating the apparatus should be as low as possible in lead, since, as observed by PRIOR (IV.), this latter metal injures yeast considerably.

§ 268. Behaviour of Yeast Cells toward Alcohol.

From the standpoint of the œcological theory of fermentation, the alcohol produced by yeast should be regarded as a weapon capable of hindering the appearance of other fungoid competitors in saccharine nutrient media. However, when accumulated in the medium during the progress of fermentation, it also restricts the further development and action of its producer. In this case, as with yeast poisons in general, the first result is the cessation of cell reproduction, a larger quantity of alcohol being necessary to arrest fermentation, and a still further quantity to kill the cells. On this point again it is impossible to expect absolutely applicable figures, because, as in other cases, the quantity of alcohol requisite for producing a given effect, even with one and the same species of yeast, is determined by external conditions (*e.g.*, composition of the nutrient medium) that cannot be closely gauged.

The restriction of cell production is effected by a quantity of alcohol that is smaller in proportion as its increase in the medium is gradual, so that the cells have been subjected to its adverse influence through many generations. Thus, according to M. HAYDUCK (VII.) the reproduction of yeast cells in fermenting distillery wash becomes sluggish when the alcohol content reaches 2 per cent. by volume, and ceases when 6 per cent. is attained. This end point coincides approximately with the appearance of ebullient fermentation, a phenomenon well known to the practical man, and one that ordinarily appears about thirty hours after pitching. From this point onward, reproduction proceeds very slowly if at all, and therefore the maker of pressed yeast gathers his crop during that preliminary stage, since the main point with him is the amount of yeast produced, and the provision of suitable conditions for reproduction his chief care, the amount of alcohol formed being a minor consideration. On the other hand, the conditions are reversed in distilleries, the object there being to minimise cell reproduction, since this goes on at the cost of the sugar in the wash and therefore of the production of alcohol. In low-fermentation breweries also the end of the cell-reproduction period is indicated by the characteristic appearance of a "head" on the surface of the wort. During this initial period the alcohol content of wort reaches 2–2.2 per cent., as was determined by MOHR (II.) and confirmed by F. SCHÖNFELD (I.).

When it is desired to prevent the development of active yeast cells in a liquid that is as yet free from alcohol—for example, to preserve wine must in an unfermented condition—the quantity of alcohol to be added, in order to produce this effect, must be larger than the 6 per cent. referred to above, and according to HAYDUCK (III.) and E. LAURENT (VI.), at least 10 per cent. is necessary. And even this limit must be raised considerably, for H. MÜLLER-THURGAU (III.) not only confirmed the observation of earlier workers that the various races of yeast differ in their degree of sensitiveness toward alcohol, but also discovered races that are capable of active reproduction in presence of 12–12.5 per cent. of alcohol in the nutrient medium. This discovery, which was made chiefly in connection with German and Swiss wine yeasts, was afterwards extended and confirmed by the researches of C. FORTI (I.) on Italian wine yeasts. In investigating the preparation of the rice spirit, Awamori, in the Loochoo Islands, near Formosa,

Inui (I.) described a yeast, *Saccb. Awamori*, which plays an active part in the process, and whose development is not crippled until the medium contains 13 per cent. of alcohol, 20 per cent. being required to arrest it completely. The Saké yeast examined by K. Yabé (II.) continues to grow until the alcohol content of the medium attains 24 per cent. A far lower power of resistance is exhibited by two red budding fungi discovered by Yabé (IV.) in the air of Japan and on the surface of rice straw, to which he gave the hardly appropriate names, *Saccharomyces Japonicus* and *S. Keiskeana*. The development of both these organisms is checked by 7 per cent. of alcohol in the medium. The budding fungi described by Richard Meissner (II.), which are incapable of forming alcohol and therefore do not belong to the yeasts, though they are of technical importance on account of their power of turning must and wine ropy (*see* p. 129), cease to reproduce and to form mucus when the medium contains over 5 per cent. (vol.) of alcohol.

Under otherwise equal conditions a larger quantity of alcohol is necessary to check fermentative activity than to stop reproduction. Thus a pressed yeast examined by Hayduck (IV.) was able to set up active fermentation in a saccharose nutrient salt solution containing 7 per cent. of alcohol, though not to reproduce therein. According to the researches of Brefeld (XV.), the addition of 17.3 per cent. of alcohol to the nutrient solution is necessary for preventing fermentation, though in Hayduck's experiments (III.) this result ensued in presence of 15 per cent. of alcohol. U. Peglion (II.) found a yeast still actively engaged in the secondary fermentation of an Italian wine containing 14.3 per cent. of alcohol. Further reports on the influencing of alcoholic fermentation by the presence of alcohol will be found in chapter lxiii., and the same point in connection with *Mycoderma* is discussed in chapter lx.

Temperature is the first of the external conditions exercising a determinating influence on the amount of alcohol required to restrict fermentation. This influence is progressive as the temperature rises within the limits that can be taken into consideration. In the first place, the rise in temperature is accompanied by a greater physical permeability of the cell membrane for the osmotic conveyance of alcohol into the cell; and, secondly, by the physiological mobility of the molecular groups of the plasma, and consequently the sensitiveness of this substance to external influences. For definite information on this point we are indebted to H. Müller-Thurgau (XIV.), who found that, under otherwise identical conditions as regards the composition of the nutrient solution and the race of yeast, fermentation was arrested

At 36° C.	as soon as	the	alcohol	content	reached	3.8	per cent. by weight.
27°	"	"	"	"	7.5	"	"
18°	"	"	"	"	8.8	"	"
9°	"	"	"	"	9.5	"	"

Practical brewers would seem to have long recognised the existence of some relation between temperature and the degree of sensitiveness of yeast toward alcohol, since they laid it down as an axiom that, in order to obtain an active sedimental yeast as well as a good beer, the temperature in the fermenting tun should be allowed to rise at the start (when the amount of alcohol present is small) and be afterwards gradually reduced.

The composition of the nutrient solution also influences the sensitiveness of the yeast toward alcohol, and therefore affects the amount of alcohol that can be produced in the fermenting liquid. Unfortunately, there is little reliable information available on this point, though the researches of H. Müller-Thurgau (XIV.) have shown that, under otherwise identical conditions, the retarding effect of alcohol increases with the amount of sugar in the medium.

This fact (the cause of which is still undetermined) is utilised in certain fermentation industries, notably in molasses distilleries. In countries where the excise duties are levied, partly or entirely, on the dimensions of the mash tun or fermenting vessel, the interests of the distiller lead him to work with mash of the highest possible concentration. To overcome the difficulty arising from the increasing sensitiveness of the yeast to alcohol in these strong mashes, prolonged tentative experiment has led to the mash being pitched at the highest gravity found to be compatible with regular fermentation, and replenished with concentrated mash in proportion as the sugar is consumed. In distilleries where raw grain (maize, &c.) or potatoes are used this method is impracticable, owing to reasons which need not be discussed here; but in breweries it is not infrequently resorted to, especially when the amount of pitching yeast is insufficient for a brew and must be increased quickly. In the strong musts intended for the production of choice wines, and fermented without this artifice, fermentation comes to a standstill before the whole of the sugar is consumed, and the resulting wines, though completely fermented, are sweet.

Yeasts in general are capable of a certain degree of habituation in respect of alcohol, so that they can be gradually accustomed to work in a nutrient solution containing a larger proportion of alcohol than was previously sufficient to arrest their activity. However, as was shown by E. Laurent (VI.), for a series of beer and wine yeasts, this is possible only within comparatively narrow limits.

H. Müller-Thurgau (XIV.) has shown that yeast cells in a nutrient medium in which the alcohol produced by their own activity gradually reaches menacing proportions, assume the condition of resting cells (*see* p. 86, vol. ii.) which remain at the bottom of the liquid, and are incapable of setting up fermentation when transferred to a fresh, non-alcoholic nutrient solution. Under these latter conditions, however, they produce in a short time daughter cells, which effect the fermentation of the proffered sugar. The prevention of this formation of resting cells is the main cause of the beneficial effect resulting from the practice of stirring up the yeast with a stick, &c., when fermentation grows sluggish in the must. When fermentation has reached the stage at which the wine begins to clarify by the deposition of the yeast, the consumption of sugar and formation of alcohol are effected almost entirely by the sedimental yeast collected at the bottom of the vessel. This alcohol, however, diffuses with considerable difficulty in the already strongly alcoholic liquid, its buoyancy being insufficient to overcome the frictional resistance. Hence a stratum, rich in alcohol, is formed immediately above the sedimental yeast, the upper cells of which are converted, under its influence, into the resting condition, and constitute a wall separating the active yeast from the still saccharine liquid, so that fermentation is arrested. The object of the stirring is to alter this state of things and remix the yeast with the liquid. The researches of J. Wortmann (VII.) on the yeast content of bottled wines confirm the above-recorded observation of Müller-Thurgau, the presence of budding fungi (yeasts and torulæ), capable of reproduction, having been detected in twenty-eight out of fifty-four samples of wine of guaranteed age in bottle.

The discovery and use of yeasts possessing high powers of resistance and low sensitiveness toward alcohol has proved especially useful to makers of wine and fruit wine; in the first place for artificially incited secondary fermentation, then for re-fermentation, and, finally, for the production of champagne (*see* p. 157, vol. ii.). Similar interest attaches to the preparation of Saké, the Japanese rice wine, which, according to A. Schrohe (II.), usually contains 14 per cent. (by weight) of alcohol, and occasionally even 18 per cent.

The foregoing particulars relate exclusively to ethyl-alcohol. With regard to the influence exerted on the fermentation activity of yeast by the homologous

allies of this alcohol, certain experiments were instituted, first by P. Regnard (I.) with an unspecified yeast in 1889, and then in 1897 by K. Yabé (III.) with the Saké yeast mentioned on p. 176, vol. ii. The former used for each 10 grms. of yeast 250 c.c. of an 8 per cent. solution of grape sugar, *i.e.*, a not particularly favourable nutrient medium. No fermentation ensued in the cultures when treated with the following additions (per cent. by volume):

	Methyl-A.	Ethyl-A.	Propyl-A.	Butyl-A.
Regnard	20	15	10	2.5
Yabé	—	—	—	—

	Isobutyl-A.	Amyl-A.	Hexyl-A.	Capryl-A.
Regnard	—	1.0	0.2	0.1
Yabé	3.0	1.0	—	0.5

Hence the toxic properties of these alcohols increase with the number of carbon atoms in the molecule.

§ 269. Inorganic Acids and Salts.

Though several observations have been published already with regard to the influence of carbon dioxide on the yeast cell, the importance of this hitherto imperfectly appreciated question to fermentation technology renders further investigation highly desirable. On repeating in 1887 the experiments first performed by C. Prandtl (I.) in 1865 on the cultivation of beer yeast in open and sealed tubes respectively, G. Foth (I.) observed a smaller increase of the sowing under the latter conditions; but it is doubtful in how far this is due to the presence of carbon dioxide or to the lack of oxygen. After L. Lindet (III.) had made further experiments, in 1889, without any decisive result, H. Ortloff (I.) in 1900 recorded a similar adverse effect on reproduction in pure cultures of *Sacch. cerevisiæ I.*, Hansen, *Sacch. Pastorianus I.*, *II.*, *III.*, *Sacch. ellipsoideus I.* and *II.*, Saaz yeast, Frohberg yeast and Logos yeast, traversed by a current of (presumably oxygen-free) carbon dioxide throughout the whole period of reproduction. With regard to wine yeasts, H. Müller-Thurgau (II. and III.) had already shown in 1889 that reproduction was checked by a high content of carbon dioxide in the wine freshly inoculated with that yeast. In the experiments of Lopriore (II.) with the cells of a hanging-drop culture of pure yeast, it was found that budding continued in a few cells during the first 4–6 hours of passing oxygen-free carbon dioxide through the Böttcher cell, but not afterwards. The divergent sensitiveness of the various species and races had already been observed by Foth (II.), who found *Sacch. Pastorianus I.* to be more resistant than Carlsberg bottom yeast No. 1; and this divergence is also deducible from Ortloff's results. A high percentage of the gas in question also diminishes the fermentative activity, judged as a whole, in the culture, this being confirmed by both Foth and Ortloff. As pointed out, however, by E. C. Hansen (XXXVI.) and J. C. Holm (I.), such a result admits of two opposite interpretations; for though the yield of alcohol per cell of yeast crop may work out lower in the cultures treated with carbon dioxide, and thus lead to the conclusion that an adverse influence has been exerted by that gas, the exact opposite may have occurred in reality, and the fermentative activity of the effective cells may have been even stimulated, especially when a number of cells in the crop became inoperative at a very early stage. Little is known with regard to the effect on the capacity of the individual cells. When working in a nutrient medium contained in a hermetically sealed vessel of sufficient strength (*e.g.*, a champagne bottle), the yeast and its fermentative activity are soon brought under the influence of carbon dioxide at high pressure, and will

suffer injury, not through the effect of the high gas pressure *per se*, toward which yeast is not very sensitive, but owing to the increased concentration of the acid in the fermenting liquid. It is desirable that the pressure at which fermentation is finally suppressed in closed vessels should be more accurately determined, the limit not having been reached at 12.6 atmospheres in the experiments made by C. G. Matthews (III.) with Burton yeast in beer wort. Conversely, the artificial removal of pressure from the fermenting liquid, by drawing off the carbon dioxide (and other volatile injurious products) accelerates fermentation and heightens the attenuation (*see* chapter lxiv.), a point already observed by Boussingault (I.), followed up subsequently by Prior (II.) and others, and finally evolved by Grauaug and Kranz (I.), by Nathan (in his Hansen apparatus), and by Pfaudler into a vacuum fermentation process which has been reported on by L. Aubry (III.).

The most important points in connection with the influencing of yeast by sulphur dioxide, whether in the gaseous state or in the form of acid salts—especially calcium bisulphite—have already been discussed on p. 85, vol. i.

Though the comparatively slight action of arsenious acid and its potassium and sodium salts on yeast—investigated by C. Wehmer (VII.) and C. Knoesel (I.)—is of no importanee in practical fermentation, it has proved useful in the theoretical study of the enzyme of alcoholic fermentation (*see* chapter lxiii.).

Hydrochloric acid, sulphuric acid, hydrofluoric acid, and certain other fluorine compounds have a relatively stronger effect on most bacteria than on yeasts, so that, by selecting an appropriate degree of concentration, the latter organisms can be protected against the former (*see* vol. i. p. 191). C. Knoesel (I.) has shown that an acid nutrient medium is not unconditionally essential to yeasts; but, within certain limits, the presence of free acids stimulates both reproduction and fermentative activity.

That boric acid has little adverse influence on yeast was observed by J. Mattern (I.) and E. Biernacki (I.); and in H. Will's experiments (I.) it proved unable to kill the whole of the cells, even after an exposure of 20 minutes. The same has been found in respect of calcium borate, and also—contrary to a previous report by Wernke (I.)—of borax, so that use can be made of another property of this substance, as already mentioned on p 131, vol. ii.

Though corrosive sublimate has been shown by Wernke (I.) and Wehmer (VII.) to have but a separatively slight toxic effect on yeast, its otherwise strongly poisonous properties render it unsuitable for use in practical fermentation. Other substances also devoid of practical importance are: bismuth nitrate, zinc sulphate, zinc chloride, ferrous sulphate, ferrous chloride, manganous chloride, potassium permanganate, aluminium sulphate, aluminium chloride, and potassium alum, the influence of which on yeast was studied by H. Will (I..)

As shown by J. Dumas (V.) and subsequently more closely investigated by U. Gayon and E. Dubourg (IV.), a considerable amount of cell juice, rich in nitrogen, can be extracted from yeast cells by exposure to the influence of a saturated solution of a suitable salt, such as sodium acetate, phosphate or sulphate, potassium acetate, oxalate, tartrate or iodide, magnesium sulphate, calcium chloride, &c. A. Béchamp (VIII. and XI.) obtained a still higher yield by kneading pressed yeast with the powdered, dry salts, an almost immediate liquefaction of the pasty mixture occurring in most cases. Saccharose is also suitable, when used in the proportion of two parts to three of yeast, and the same applies to gum arabic, &c. The auto-fermentation frequently occurring under these conditions was investigated by C. J. Lintner (III.), in a series of experiments to which further reference will be made in chapter lxv.

§ 270. Organic Stimulants and Poisons.

The mutual relations between yeasts and the organic acids of the aliphatic series are varied. Some of them, succinic acid, for instance, occur as decomposition products of the cell substance (*see* chapters lxiv. and lxvi.). In other cases similar acids play the part of a source of carbon, and therefore supply material for the process of metabolism, as already mentioned on p. 150, vol. ii.; whilst in still other instances, with which we shall now deal, they excite interest on account of their stimulant or poisonous action. Yeasts are very susceptible to butyric acid, and must therefore be protected from the danger with which they are menaced by this acid in distillery work (*see* vol. i. p. 188). The general rule that yeasts of different species are variously influenced by any given stimulant has found practical utilisation in connection with tartaric acid. On the occasion of his critical examination of Pasteur's method of purifying the pitching yeast used in the brewery, E. C. Hansen (XXXIV.) found that the beer yeasts, *Sacch. cerevisiæ I.*, Hansen, Carlsberg bottom yeast No. 2, &c., are more susceptible to tartaric acid than the wild yeasts (*Sacch. Pastorianus I.* and *III.*, *Sacch. ellipsoideus II.*); so that in a mixture of the two classes the relative proportion changes in favour of the wild yeasts when the culture is grown in a 10 per cent. solution of saccharose containing 4 per cent. of tartaric acid. The progressive diminution of the culture yeasts and increased percentage of wild yeasts obtained by successive reinoculations in such a liquid, forms an excellent means of detecting the latter in critical cases. The Saké yeast examined by K. Yabé (III.) will not develop at all in this solution. The influence of acetic acid was first investigated, in the case of pure cultures, by Lafar (II.), who found that the fifteen species of wine yeast examined differed considerably in point of sensitiveness. Roderich Meissner (I.) afterwards found the same result in the case of Saaz, Frohberg and Logos yeasts, which, however, cannot stand nearly so much of this acid as the wine yeasts in question, the first two losing their fermentative capacity almost completely in presence of 0.25 per cent., and the third with 0.375 per cent., whereas all the fifteen wine yeasts continued to ferment in presence of 0.78 per cent. of acid, and three of them even with 1 per cent. H. Müller-Thurgau (XXI.) found that the restrictive influence of this acid on development can be ameliorated by aerating the nutrient medium (must). According to Duclaux (I.), the presence of 0.4 grm. of formic acid per litre will retard the development of cells of various kinds of beer yeast sown in wort, reproduction ceasing entirely when the addition is doubled. Oxalic acid is found by O. Loew (IX.) to destroy the fermentative power of yeast in twenty-four hours, when forming 1 per cent. of the solution; and H. Will (I.) states that the same result ensues with 10 per cent. in five minutes. In the case of succinic acid, M. Hayduck (VIII.) stated that even 0.59 per cent. does not hinder fermentation by yeast; and E. Kayser (X.) showed that this quantity is consumed by the organism. The same worker (IX.) also found variable degrees of sensitiveness to malic acid and citric acid on the part of different yeasts. In the comparative tests made by J. Behrens (VIII.) with Carlsberg bottom yeast No. 1 in unhopped beer wort, an addition of 0.2–0.4 per cent. of citric acid retarded the maximum development of fermentative capacity to some extent, without diminishing the total effect.

The behaviour of yeasts toward hop resins—a chemical bibliography of which has been compiled by G. Barth (I.)—has not yet been sufficiently investigated. Three of these resins (*see* vol. i. p. 93) were isolated from hops by M. Hayduck (IX., X., XII., I.), who showed that no appreciable antiseptic effect is produced

by hop tannin or by the ethereal oil which imparts to hops their characteristic aroma. On the other hand, according to this worker, the two soft resins decidedly retard the progress of fermentation; but L. AUBRY (III.) proved that the final attenuation in hopped wort is higher than in unhopped wort, a result confirmed by J. BEHRENS (VIII.), though F. W. RICHARDSON (I.) observed the contrary. Further attention should be devoted to Hayduck's observation that the amount of nitrogen compounds absorbed by yeast from wort increases with the quantity of hops employed, bearing in mind a statement made in this connection by BEHRENS (VIII.). With regard to the part played by hop resins in the formation of "head" on the fermenting wort, compare p. 133, vol. ii.

Many yeasts are rather sensitive to the tannins in wine must and certain fruit musts, as was observed by A. ROSENSTIEHL (I.). This is a well-known fact among vintagers, and special measures are adopted in consequence.

The nature of the resins and ethereal oils rendering the fermentation of juniper-berry juice a difficult operation is still unknown. G. KASSNER (I.) published a note on this point.

According to WERNKE (I.), oil of mustard ($C_3H_5.NCS$) in the proportion of 1 : 16,700 is fatal to yeast.

The maltol ($C_6H_6O_3$) first discovered by J. BRAND (III.) in caramel-colour malt, and regarded by KILIANI and BAZLEN (I.) as a methylpyromeconic acid, is stated by H. WILL (XXVI.) to have but a feeble toxic effect on yeast and to be devoid of influence in practice, for though 0.1 per cent. will delay yeast reproduction, the amount present in wort is far below that proportion. Even the furfural ($C_5H_4O_2$) produced during the curing of malt (*see* p. 151, vol. ii.), from which it passes into the wort—though rarely found in beer—is stated by H. WILL (XXVII.) to have but little effect on yeast, though the different yeasts are variously affected and all are killed by an addition of 0.5 per cent. It has not yet been definitely settled whether and to what extent this or other products of the curing of malt are responsible for the admitted fact that dark worts furnish a lower attenuation than those from pale malts. Researches on this point were undertaken by M. IRMISCH (I.) and F. Niemeyer.

Among the compounds of the aromatic series, WERNKE (I.) states that benzene (C_6H_6), toluene ($C_6H_5.CH_3$), and xylene ($C_6H_4(CH_3)_2$) are fatal to yeast in the proportions of 1 : 200, 1 : 300, and 1 : 800 respectively. The action of carbolic acid or phenol ($C_6H_5.OH$) on yeasts was studied by Lemaire, W. BUCHOLZ (I.), H. HOFFMANN (V.), and H. FLECK (I.), and afterwards more carefully by C. KNOESEL (I.), who found that an addition of about 0.5 per cent. of phenol, at room temperature, kills the cells. According to BIERNACKI (I.) the introduction of a second and third hydroxyl group into phenol lowers its toxic properties, so that resorcin ($C_6H_4.(OH)_2$) is only half as powerful, and pyrogallol ($C_6H_3(OH)_3$) has only one-third the strength of phenol. K. YABÉ (V.) confirmed this observation, and found it also applicable to pyrocatechin, hydrokinone, and phloroglucin. Benzoic acid, even in the small proportions in which it is soluble in aqueous liquids, has a somewhat powerful effect on yeasts, according to the discovery made by H. WILL (I.) and (at a later date) by C. WEHMER (VII.), and confirmed by H. FLECK (I.) This is utilised in the practice of preserving fruit (*see* vol. i. p. 91). Comparative tests made by WEHMER (XV.) on Frohberg yeast with benzoic acid and its three monoxy-derivatives, showed that 0.1 per cent. of the first-named and also of its ortho-derivative (salicylic acid) entirely prevent the development of yeast, though the m- and p-oxybenzoic acids in the same concentration have no appreciable influence. According to G. HEINZELMANN (VI.), 0.1 grm. of salicylic acid per litre has a stimulating rather than an injurious

action (*see* vol. i. p. 88), whereas 0.37 per cent. is fatal to yeast; and similar results were obtained by H. Will (I.). The behaviour of yeast toward cinnamic acid was reported on by Fleck (I.). Saccharin, which was found by Burkard and Seifert (I.) to have little effect on yeast, is stated by Macheleidt (I.) to prevent fermentation in hopped wort when used in the proportion of 1 per cent. No appreciable influence in this connection could be detected by G. Bryilants (I.) with 0.7 per cent. of phenolphthalein. The behaviour of yeasts toward certain alkaloids was investigated by Cl. Fermi and E. Pomponi (I.).

SECTION XIV.

LIFE HISTORY AND VARIABILITY OF THE SACCHAROMYCETES. CLASSIFICATION OF THE SACCHAROMYCETES AND SCHIZOSACCHAROMYCETES. By ALBERT KLÖCKER, Copenhagen.

CHAPTER LIII.

THE LIFE HISTORY OF SACCHAROMYCETES IN NATURE.

§ 271. Fundamental Researches on the Life History of Saccharomycetes.

The first of the yeast fungi whose cycle of existence was traced in nature was the *Saccharomyces apiculatus*, more fully described in chapter lxi., the researches having been carried out by Hansen. This small, lemon-shaped asporogenic alcohol yeast, which is widespread in nature and generally known, received its name, *Sacch. apiculatus*, from Reess. In the following lines the term Saccharomycetes, whether used in a general sense or qualified by the prefix "true," will be applied to alcohol yeasts capable of producing endospores as well as of budding. As the researches in connection with *Sacch. apiculatus* are typical of those concerned with the life history of the true Saccharomycetes, a brief introductory *résumé* of them will now be given.

In the years 1880–1881 Hansen (XXII. and IX.) published the results of his investigations on the career of the yeast in question. From these it appears that the chief habitat and breeding-place of this fungus in the summer and autumn are damaged, sweet, juicy fruits, in the juice of which it reproduces in great abundance, whilst in winter and spring it inhabits the soil underneath fruit trees and bushes. From the fruit it finds its way into the soil, either through the dropping of the fruit or the swilling action of rain, as well as in the excrement of the numerous insects that infest and devour the sweet, juicy fruit inhabited by the cells. With regard to the means by which the minute cells are transported from the soil to the fruit, Hansen ascribes the principal *rôle* to the wind; though, both in his first and subsequent treatises he remarks on the great importance of rain, insects and other animals in this connection, the transference of cells from one fruit to another being attributed to insect agency. A powerful storm of rain may splash up the wet soil, accompanied by the yeast cells, on to the fruit of low-lying plants, such as strawberries. Whilst the action of insects as conveyers of infection is restricted to a short period in the year—not only in the vicinity of Copenhagen, where Hansen's researches were carried on, but also throughout the greater part of Europe—the wind continues to act all the year round, enormous numbers of the cells being carried up in clouds of dust and deposited on the fruit. The researches also show that this life history is the normal one for the yeast in question, and that the cells soon die when deposited on unripe fruit, owing—as was ascertained by Hansen (X.)—to the fact that the fungus has a very low power of resisting drought and the action of the sun's rays.

We will now turn to the question of the life history of the true *Saccharomycetes*. When Hansen commenced his investigations in this direction there had already been published a series of researches by Brefeld and Pasteur running contrary to the ideas which formed his starting-point.

Brefeld (XVII.) formed the opinion that yeast cells not only reproduced in the alimentary canal of the animal organism, but that their chief breeding-place and habitat was the excrement of herbivorous animals. Hansen showed that this is incorrect.

From the time of the first researches on the fermentation of wine, it was a recognised fact that ripe—and especially damaged—grapes are rich in yeast cells at gathering time, and it naturally followed that the yeast cells would find their way into the soil with the fallen grapes or when swilled off by rain. Pasteur (XXX. and XVIII.), however, concluded from his experiments that the cells could not live long in the soil, and that the latter therefore could not form their winter habitat, though he gave no hint as to what actually constitutes the latter.

Hansen (LVII.) adopted two methods in his researches: partly the analysis of samples of soil and other natural substrata, including the dust in the air, and partly the sowing of certain species in the soil under natural conditions. The results showed that true Saccharomycetes are to be found in the soil and the air at all periods of the year, but most abundantly when the sweet, juicy fruits are ripe. The inoculation experiments were performed with *Sacch. cerevisiæ*, *Sacch. ellipsoideus* and *Sacch. Pastorianus*, the yeasts being sown in sterilised soil, placed in flower-pots embedded in the ground out of doors. Here it was proved beyond dispute that the cells live from one fruit harvest to another; and at the same time the observation was made (LVIII.) that the cells are capable of producing endospores on the surface of the ground. On the basis of these experiments Hansen was able, in his second communication in 1882, to demonstrate that the life history of the true *Saccharomycetes* is substantially identical with that of *Sacch. apiculatus*. The chief breeding-place is on sweet, juicy fruit, the soil constituting the winter habitat, whilst the principal methods of transport are the wind, rain, insects and other small animals.

The inoculation experiments were repeated subsequently by Hansen (LIX. and XLIII.) both with *Sacch. apiculatus* and with, in part, the same *Saccharomycetes* (viz., *Sacch. ellipsoideus*, *Sacch. Pastorianus*, Carlsberg bottom yeast No. 1 and a top-fermentation beer yeast), but in this case the flower-pots were replaced by Chamberland earthenware pipes, in order to ascertain whether the yeasts could survive for several years in the soil. The earthenware pipes were used in order to protect the cells, as far as possible, from infection and the ravages of animals from the surrounding earth. In the result it was found that the yeasts in question are able to live for more than three years in soil.

Both at that time and subsequently, the question of pleomorphism in these fungi was actively discussed, especially in consequence of the researches of Brefeld, and Pasteur's theory of the development of Saccharomycetes from brown *Dematium* cells must be classed in the same catagory. It was considered possible that still living original forms of these yeast fungi might be discovered, and probably of such a character as to point to a very different life history to that established by the researches on *Sacch. apiculatus*. Hansen himself, by referring to this possibility in several of his later publications, not only led to the following up of this line of research by several workers, but also to the institution of experiments by others with a view to upsetting the theory he had commenced to establish in connection with the life history of yeasts.

The repetition of Hansen's experiments, especially in connection with the analysis of vineyard soils, became a matter of practical and theoretical import-

ance; and in 1889 a series of investigations in this direction was undertaken by H. Müller-Thurgau (XXIX.). This worker, also, found that the chief breeding-place of the Saccharomycetes is on fruit, and that the cells of wine yeasts can be discovered in soil all the year round. His experiments were made in a vineyard at Geisenheim, and he was the first to ascertain how deep yeast cells can be embedded in the earth and continue to live, namely, 8–12 inches on the average, none being found as deep as 16 inches. In the summer time the number of yeast cells on the surface is smaller than at a depth of a few centimetres. At the outset, Müller-Thurgau held the opinion that insects formed the chief means of transporting the yeast cells, and he declined to admit the importance of wind in this connection. Hence the only point on which he was in complete accord with Hansen was that, also in vineyards, the soil forms the normal winter habitat of the Saccharomycetes, and the latter find their way thence to the breeding-place, namely, the grapes. Later on, however, he recognised (LVIII.) that both wind and rain are important means of transport. His experiments with sowing wine yeasts in earthenware pipes showed that the fungi can live in the soil from one autumn to another. Müller-Thurgau also states that *Sacch. ellipsoideus* has a very low power of resisting drought, and therefore soon dies when on the surface of grapes exposed to very dry weather and intense sunlight—an observation also confirmed by Martinand (II.).

A few years later a thorough investigation of vineyard soil was undertaken by Wortmann (VI.), with samples taken from the same plot at intervals of fourteen days during two years. The largest number of yeast cells was found in November and December; and must inoculated with the samples of soil quickly began to ferment. During January, February and March the number of yeast cells diminished; and during the spring and summer—the latter especially—the proportion became more and more unfavourable, and the yeasts disappeared from a progressively larger number of the samples. The most unfavourable results were obtained in late summer: August and September; but from the beginning of the grape harvest the conditions improved almost immediately. Wortmann's conclusion was to the effect that the yeast cells become enfeebled during their sojourn in the soil, most of them dying off; and that the continuation of the species is confined to the few cells that survive the winter and are fortunate enough to find themselves on a damaged grape. As will be evident, especially from what follows, this does not entirely coincide with Hansen's experiments, according to which the conditions in the soil are not so unfavourable for the *Saccharomycetes*. With regard to the means of transport, he thinks that Hansen gives undue credit to the wind, his own experiments tending to show that the chief part is played by wasps.

Whilst both Wortmann and Müller-Thurgau agree in the main with Hansen's theory on the life history of the *Saccharomycetes*, the workers named below express a different view on certain points, several of them stating that in hot climates, Italy in particular, the soil is not the chief habitat of the yeast cells.

Boutroux (IX., X.) regards the nectar of flowers, insects, and unripe fruit as constituting the habitat of yeast fungi from the end of winter to the fruit season, and states that the cells are conveyed from flower to flower and from fruit to fruit by insects. It must, however, be remembered that he makes no distinction between *Saccharomyces* and *Torula*, but applies the former name to all yeast cells capable of inciting fermentation. Probably, therefore, the cells found by him were not always *Saccharomycetes*, most of them being certainly forms of *Torula*, which are widely met with in nature. Neither Hansen (LIX.) nor Beijerinck (XXVIII.) was able to confirm Boutroux's communication. Another remarkable result obtained by this worker (III.) was that insects play a more

important part than wind in the conveyance of yeast cells that are unable to invert saccharose, the converse being the case with yeasts capable of inversion.

Rommier (I.) is of opinion that *Sacch. apiculatus* passes the winter in honeycomb; but neither these nor any other yeast cells have been discovered in comb by Boutroux, Hansen, Beijerinck, or Klöcker. Of course it is possible that a few isolated yeast cells or other micro-organisms may be found occasionally in any situation; but we are now concerned solely with large quantities and constancy of occurrence.

Berlese (IV.) asserted that the digestive canal of insects forms the true winter resort of the *Saccharomycetes*, and that Hansen was in error when he located this resort in the soil; and he claimed that the cells pass the winter in flies (especially in Italy). This statement may, however, be disregarded, since the insects do not themselves pass through the winter in the perfect state (imago) in Europe, except in the most southerly districts (and therefore not at all in the largest part of Italy); in addition to which he only succeeded in finding a single fly containing *Saccharomyces* among 150 examined. He also states that the cells pass the winter in ant-runs in hollow trees and woodwork—a circumstance of no importance even if true, because these habitats are so rare in comparison with the area presented by the soil that even if every ant's dwelling contained *Saccharomycetes* their number would be insignificant in comparison with those found in the soil. Moreover, Klöcker carried on a large number of experiments on the behaviour of insects toward *Saccharomycetes*, and found that insects are devoid of importance as a winter habitat, at least in Europe north of the Alps.

That *Saccharomycetes* are able to pass without injury through the alimentary canal of various animals has been demonstrated by several workers, *e.g.*, by Klöcker and Schiönning (VIII.) in the case of insects and birds; by Berlese (IV.) with insects; and by Cordier (I.) in the case of insects and animals. Consequently the micro-organisms in question may also be disseminated by these means.

§ 272. Later Experience on the Life History of Saccharomycetes.

The starting-point and basis of the foregoing researches on the life history of the true *Saccharomycetes* were constituted by the results of Hansen's experiments on *Sacch. apiculatus*. However, though the Hansen theory sufficed to explain all the observations made with respect to that species, it was, in several cases, not unconditionally applicable to the true *Saccharomycetes*. Thus, in his researches in the German wine districts, he found that at certain periods the samples of soil from the vineyards contained fewer yeast cells, and also wine-yeast fungi, than similar samples from adjacent meadows. Indeed some 50 c.c. samples of vineyard soil did not contain a single living cell that could be identified as *Sacch. ellipsoideus*. Analogous, though scarcely so extensive, irregularities occurred in certain analyses of orchard and arable soils in the vicinity of Copenhagen. Consequently, Hansen (LX.) was led to extend his researches and proceed in a different manner, the methods being made more stringent, the number of the analyses greatly increased, and the area of the experiments broadened to comprise an enormous district, from Scandinavia to southern Italy, from the plain to the highest mountain top. The principal new direction taken by these experiments was, however, the investigation of the secondary habitats. The analyses of soils from round Copenhagen showed that true *Saccharomycetes* are to be found everywhere in the soil all the year through, even in places where *Sacch. apiculatus* is only detected occasionally if at all, that is to say, at considerable distances from fruit gardens. Only when the number of analyses had reached sufficient dimensions did it appear that the garden soils are the richest in

Saccharomycetes, and that the number of the cells diminishes as the distance from these centres increases. For instance, in a series of 200 analyses, true *Saccharomycetes* were found in 67 per cent. of samples of soil taken from under fruit trees and fruit bushes; in 30 per cent. of those from under deciduous and coniferous trees in the vicinity of fruit gardens, and only on 19 per cent. of samples from distant fields. Similar results were obtained in the experiments conducted in mountain districts, *e.g.*, the Hartz Mountains and the Alps. The greater the altitude and the distance from fruit gardens, the less plentifully are *Saccharomyces* cells found in the soil. Hansen's newer analyses show that the parallel also holds good in warmer climates, *e.g.*, Italy.

The reason of the presence of *Saccharomycetes* at considerable distances from fruit gardens and primary breeding-places in general is in part traceable to the fact that their power of producing endospores makes them better fitted than *Sacch. apiculatus* for resisting drought. On the other hand, it is to some extent due to their higher capacity for reproducing in nature in numerous secondary breeding-places, apart from the primary ones (sweet, ripe fruit), the latter, moreover, being located in woods and other places, and not merely restricted to gardens and vineyards. Such secondary breeding-places are formed by the liquid matters of the soil, *i.e.*, organic extracts of animal and vegetable substances, manure, &c. True, the reproduction effected in this way is very small in comparison with that of the primary breeding-places, and especially so far as *Sacch. apiculatus* is concerned, this species being reproduced under these conditions less extensively than the true *Saccharomycetes*. In addition, the latter will also stand a longer immersion in water than *Sacch. apiculatus*.

Saccharomycetes are found not only on the surface of the ground, but also in the thin layers of soil found above ground on trees, brickwork, stones, &c., where they are protected from drought by a stratum of moss, lichens and algæ. These plants avidly absorb water, and the under layer—which, especially in the case of moss, consists of dead residual matter—readily cedes nutrient substances to the water. In forests of deciduous trees the foliage affords additional protection against drought; but in the open fields different conditions obtain, the *Saccharomycetes* occupying secondary breeding-places there, being exposed to more or less extensive desiccation.

These studies on secondary breeding-places and on the behaviour of the various species towards drought, furnished Hansen with an explanation of the irregularities referred to above. When *Saccharomycetes* cannot be found in the primary breeding-places (sweet, juicy fruit), sun, wind and weather have made their influence felt; and when, on the other hand, they are found abundantly in the open fields, the reason is that they have discovered unusually favourable secondary breeding-places there, and have at the same time been protected from desiccation. The secondary breeding-places are of considerable importance by reason of their extensive distribution. An examination of the behaviour of the species towards temperature also explains in some respects what is taking place in nature, Hansen having found that several species are capable of reproduction when the surrounding temperature is at freezing-point—though under these conditions several months are necessary for the production of a single generation, even when the cells are situated in a favourable nutrient liquid. As a rule reproduction ceases when the temperature falls to 1°–2° C., and a much higher degree is necessary to enable it to proceed with vigour. For this reason the number or yeast cells found in a given spot varies according to the time of year, being greatest in the fruit season, at which season the most favourable conditions are found in respect of temperature, food-supply and moisture. Among the primary breeding-places the soil furnishes its maximum yield in autumn: and it follows from what has been stated above that the secondary

breeding-places are active at the same time. Afterwards, the fluctuations in the course of the year are considerable, more especially, as already mentioned, where extensive drying takes place.

The principal result therefore is that the soil is the chief habitat of the *Saccharomycetes* in general (as it is in the case of *Sacch. apiculatus*) all the year round. From this starting-point the cells are transported by the aid of wind, rain, insects and other small animals, to the primary breeding-places (the sweet, juicy fruits), and thence in turn by the aid of the same factors either to new primary breeding-places, where extensive reproduction occurs, or else to a more modest existence for an indeterminate period in a secondary breeding-place. In the fruit season an important part in the conveyance from one primary breeding-place to another (often over long distances) is played by birds; and, in addition, cells find their way from primary breeding-places to the soil in the excrement of these animals. At present the Hansen theory, especially since his investigations on the secondary breeding-places, affords a natural explanation of all the observations hitherto made.

The importance of these experiments to the practice of brewing consists chiefly in the light they have thrown on the habitats of wild yeasts, and on the way in which these yeasts can find their way into the brewery. Thus it is evident that atmospheric dust at all times of the year may contain cells of true *Saccharomycetes* and also those of wild yeasts. The soil of fruit gardens and vineyards constitutes the chief source of danger, especially during the season of ripe fruit. In the air analyses referred to above it was found that the greatest risk of infection by yeast cells in the brewery is greatest, for Denmark, in the months of August and September. As a rule these cells find their way into the brewery *viâ* the cooler, but they may also enter the fermenting-room direct. Where the conditions allow the cooler to be abolished, this should be done. Nevertheless, the risk of infection has been greatly diminished by the introduction of pure-culture yeast, the more or less enfeebled wild yeasts gaining access to the wort on the cooler being generally suppressed by the pure yeast in the fermenting vessel.

CHAPTER LIV.

VARIABILITY AND HEREDITY IN SACCHAROMYCETES.

§ 273. Temporary Variations.

Both the morphological and physiological characteristics are subject to variation, not only among micro-organisms but also among higher organisms, both vegetable and animal. Just as the study of the *Saccharomycetes* first obtained precision when the discovery of reliable methods of pure culture furnished a sure starting-point for the investigations, so also the era of trustworthy observations on variation dates from the same period.

We are indebted to the researches of Hansen for the foundation of our knowledge of the variations of the *Saccharomycetes*. These researches form two main groups: one comprising such variations as must be considered temporary and dependent on conditions at present unknown, whilst the other relates to variations occurring under known conditions. The experimental researches and the results obtained in connection with this latter group form the most important part of Hansen's labours in this field.

The variations may be classified into temporary and constant from another point of view, temporary variations being those continuing for only a limited time, at the expiration of which they disappear, either spontaneously or under special treatment, whilst the constant variations are those that cannot be restored to their original condition by any treatment.

We shall first discuss the temporary variations, citing different examples. The total number of these variations is naturally enormous.

Hansen's observations in this connection were commenced at the time he introduced pure culture, and they include examples from nearly every branch of the morphology and physiology of the yeast cell, the important being given below. Thus he observed that a yeast from the brewery gave a higher attenuation, defective clarification, a strange flavour, &c., after it had been grown for some time in the laboratory, but regained its original properties on being returned to practical use. This observation has also been made by other workers. Moreover he found (XL.) that when Carlsberg bottom yeast No. 1 was grown on wort gelatin, it furnished some colonies consisting of oval cells, whilst those in others were elongated and therefore abnormal in shape. Both sets of colonies when grown by themselves produced descendants which retained their characteristic shape for some time, the elongated cells only resuming their normal form after recultivation in wort for a certain period. In the brewery, also, the normal elongated form was retained during several fermentations. The asporogenic form of *Sacch. intermedius* (= *S. Past. II.*) obtained by cultivation at 25° C. on wort gelatin, produced cells which, when grown in wort cultures, furnished vegetations some of which resembled *S. ellipsoideus* and others *S. Pastorianus*, the difference persisting during a large number of cultures, both at the ordinary room temperature and at 25° C.

The clarifying power of beer yeast can be influenced to some extent by the previous method of cultivation (*see also* pp. 137, 138, vol. ii.), as was clearly shown by the experiments of Hansen (XLII.) with Carlsberg bottom yeast

Nos. 1 and 2. The cultivation of these two species separately in aerated wort furnished vegetations which clarified satisfactorily in the brewery; but when the same species were grown in unaerated wort, the resulting yeasts did not act normally in practice until they had been put through several fermentations. The No. 1 yeast reverted to its original condition quicker than No. 2, the transitory modification sustained by them both having been greater in the one case than the other. The beer obtained by the fermentation of the unaerated wort was highly opalescent, and, as a rule, little improvement in this respect was effected by prolonged storage, the beer remaining cloudy even after the yeast cells had settled down and the liquid had remained exposed to ordinary room temperature for several days. This was more particularly the case with the beer fermented with the No. 1 yeast. The subject of variation in clarifying power has also been reported on by A. Jörgensen (XII.), who observed that top yeast kept on gelatin clarified more slowly and gave a higher attenuation than when kept in wort. There is no doubt that chemical influences are concerned in this case, as may be concluded from Hansen's observation that *Saccb. Pastorianus I.*, when repeatedly grown for many generations in a solution of saccharose in yeast water at 32° C., loses for a time its property of producing the characteristic disagreeable taste and smell in wort (*see* p. 84, vol. ii.). Continued cultivation in wort, however, soon causes the vegetation to revert to its original state.

These instances of temporary variation may be supplemented by the following, also observed by Hansen (XXXVIII.). The film cells of certain species, and also cells derived from old vegetations grown in saccharose solution, gave, in wort cultures, a loose, curdy deposit, quite different from the ordinary, pasty form; but the original type was restored by repeated cultures in wort. Similar curdy yeast may be produced when the yeast has been left for some time in a desiccated condition.

In 1886 the same observer (XXXIX.) reported an experiment in which pure-culture bottom yeasts behaved like top yeast, but reverted to the original type after several re-inoculations. It was also found that typical top yeasts can behave like bottom yeasts for several generations, the whole being therefore merely transitory variations. A few instances had also been previously reported (in 1884) by Hansen and Kühle, in which stormy fermentation, with the characteristics of top fermentation, was produced at once in wort by samples of Carlsberg bottom yeast No. 2, which had been kept in the brewery for several weeks, partly in beer, partly in wort, and partly as washed, pressed yeast in an ice-chest. Even in this case, however, the vegetation quickly reverted to the original state. Similar observations have also been recorded in later years, Henneberg (IV.), for instance, having examined at the Berlin Experimental station a typical Dortmund bottom yeast which, after acting satisfactorily for some time in the brewery, at length began to form a head and deposit similar to top-fermentation yeast. Nothing could be ascertained as to the cause of this variation. According to Lindner (XXVIII.), this yeast eventually reverted to its normal state, and was successfully used in practice.

The percentage content of the enzymes present in the cells is also subject to variation, the cause being largely attributable to the method of nutrition. There is, however, no proof available that yeast can lose its capacity of producing enzymes so completely as not to regain it under favourable conditions of growth, nor is there any known instance of a yeast producing, under special treatment, a new enzyme that it previously lacked. The assumption put forward by Dubourg (I.) and other French workers, that, under suitable treatment, a yeast could be induced to form an enzyme that it had not previously produced, has been shown to be totally inaccurate by the experiments of Klöcker (IV.).

This statement does not imply the non-existence in nature of species in a state of transition in this respect, but only that all the species hitherto closely examined have proved constant in their behaviour towards sugars (*see* chap. lxv.). Recently it was found by WARSCHAWSKY (I.) that *Sacch. cerevisiæ I.* and *Schizosacch. Pombe* produce zymase only when grown on a fermentable nutrient medium, this enzyme not being formed when the medium is unfermentable. He also found that even under the former conditions, *Schizosacch. Pombe* does not produce zymase when the nitrogen in the medium is in the form of ammonium phosphate. On the other hand, zymase is again formed when favourable conditions of cultivation are restored, so that the case is merely one of temporary weakness. Moreover, no one has yet succeeded in depriving an alcohol-forming yeast of that property, nor has this tendency been found in any case of spontaneous variation.

With regard to the influence of chemical and physical factors on the production of more or less temporary variations, the reader is also referred to chapter xlvi. of the present volume.

§ 274.—Hansen's Researches on Asporogenation. The Production of Constant Varieties by Transformation.

HANSEN'S (XL.) discovery, in 1889, of asporogenation, *i.e.*, the loss of the capacity for producing spores, in *Saccharomycetes*, opened up a new stage in the investigation of variation among these micro-organisms. He observed in the case of *Saccharomycodes Ludwigii* that a number of cells lost the power of forming spores when grown for some time on one and the same nutrient medium, whilst another portion of the cells was considerably weakened in this respect; the remainder, however, remaining unaffected. This variation proved hereditary for some time in wort cultures. The same peculiarity was also observed in other species, *e.g.*, *Sacch. cerevisiæ*, *Sacch. Pastorianus*, *Sacch. intermedius* (= *Sacch. Pastorianus II.*), *Sacch. validus* (= *Sacch. Pastorianus III.*), *Sacch. ellipsoideus I.*, and several bottom-fermentation beer yeasts, when kept on wort gelatin or in wort, a larger or smaller proportion of the cells losing the faculty of producing spores. BEIJERINCK (XXII. and XXIV.) subsequently found the same behaviour in the case of *Schizosaccharomyces octosporus*. This worker also noted several points of difference between the asporogenic cells and the others, the former producing less trypsin and larger quantities of acid. Another species, which he named *Sacch. orientalis*, also revealed the existence of a relation between sporogenation and proteolysis, inasmuch as the asporogenic colonies in a surface-plate culture did not liquefy the gelatin, whilst the sporogenic cultures did. Moreover, the former cells were quite destitute of glycogen, though the sporogenic cells contained that substance. The loss of sporogenic capacity in *Saccharomycetes* stored in the laboratory is also mentioned by LINDNER (XXIX.).

In these cases the variation is partly transitory and partly constant. In certain instances Hansen succeeded in restoring the faculty of sporogenation to asporogenic cells of *Sacch. Ludwigii*, by cultivation in a nutrient medium containing dextrose. In other cases, however, both this and other culture methods proved unavailing, the cells remaining asporogenic. In this connection mention may also be made of KLÖCKER'S observation (III.), that a vegetation of *Sacch. Marxianus*, which produced only a few spores, was considerably strengthened in this respect by cultivation in a medium containing dextrin.

HANSEN (XXXVII.), in 1883, showed that spores can stand greater heat than the vegetative cells. This observation was taken by BEIJERINCK (XXII. and XXIV.) as the starting-point in his endeavours to produce vigorous sporogena-

tion in a culture originally forming only a small number of spores. There is no fixed method for this purpose, the treatment differing with the kind of culture and requiring to be performed tentatively in each case. Beijerinck made the mistake of applying the term regeneration to the result of his experiments, the matter being merely one of selection of the individuals which have not lost their sporogenic power, so that it is incorrect to speak of a lost capacity of the individual.

We will now deal with Hansen's fundamental experiments, in which he produced permanently asporogenic varieties by the action of certain external agencies, in the same year (1889) in which he observed the spontaneous asporogenic varieties of *Sacch. Ludwigii* mentioned above. In the course of his experiments on the limits of temperature for budding and sporulation in *Saccharomycetes*, he observed that the maximum temperature for the former function is always a few degrees higher than that of the latter; whilst the minimum temperature of budding is a few degrees lower than that of sporulation. This law holds good for all true *Saccharomycetes* (*see* p. 99, vol. ii.).

Hansen tried to ascertain what happens when *Saccharomycetes* are grown at temperatures intermediate between the said two maxima and minima respectively, and whether the result is the same in each case. This latter, however, does not occur, the species undergoing a remarkable change on cultivation at a temperature intermediate between the two maxima, though not in the other event. This change consists in the *Saccharomyces* vegetation completely losing the power of sporulation when cultivated for a number of generations in nutrient liquid at the temperature in question. As might be anticipated, it was also found that the maximum temperature is not exactly the same for all the individuals constituting a vegetation. In experiments for ascertaining the maximum temperature of a species, the result applies to the individuals possessing the highest maximum, though there may be also other individuals present which have a slightly lower maximum temperature. Hence modification experiments may reveal the presence of individuals which apparently can be modified at a temperature below the maximum found for sporulation, whereas in reality these individuals belong to the group exhibiting the lower maximum temperature of the species; and therefore, even in this case, the modification occurs at a temperature intermediate between the maxima for sporulation and budding respectively. Consequently it may be stated, as a general proposition, that the said modification proceeds by cultivation at a temperature bordering on the maximum temperature for sporulation. The material for these modification experiments is taken from a young and vigorous vegetation, grown in wort under ordinary conditions and at a suitable temperature. A new culture is started with this material in wort, at a temperature between the maxima for sporulation and budding, this temperature varying for different species. After this method of culture has been in progress for a short time, an average portion of the vegetation grown at the high temperature being transferred every day to a new flask of wort at the same temperature, the cultures being shaken up several times a day, asporogenic vegetations are obtained. The number of cultures necessary for producing this result varies according to the species. In this manner Hansen obtained constant asporogenic varieties of all the species that have been proved to belong to the genus *Saccharomyces;* but no modification could be produced by this treatment in the case of the genera *Pichia*, *Willia* and *Saccharomycodes*.

In order to elucidate the progress of this modification, Hansen (XLIV.) afterwards carried out special investigations, the chief result of which may be summed up as follows: The material consisted always of a single cell of the species in question; in some cases a vegetative cell, in others a spore, the material through-

out being thoroughly capable of sporulation, and in which the strictest examination failed to reveal a single asporogenic cell. To facilitate the examination of the conditions of sporulation throughout the treatment, plate cultures were prepared by transferring the cells to the surface of wort gelatin by means of a platinum stylus. The mature colonies, when of sufficient size, were transferred direct on to moist gypsum blocks for sporulation, those too small for this treatment being first placed in wort and the sedimental yeast therefrom transferred to the gypsum. The principal experiments were made partly with *Sacch. Past. I.* at 32° C., and partly with Johannisberg 2 wine yeast at 36° C. The following table gives an example of the results obtained from the former yeast during the various stages of the treatment.

In stage 2	1 per cent.	of constantly asporogenic cells	was found.
„ 4	60 „	„	„ „
„ 7	100 „	„	„ „

Each stage represents twenty-four hours.

To settle the fundamental question whether this formation of asporogenic varieties is due to selection on transformation, Hansen instituted further investigations with Johannisberg 2 yeast. In the normal vegetation it was absolutely impossible to find a single cell which did not produce sporogenic vegetations when grown under normal conditions. The vegetation employed for starting the experiment was analysed by isolating at least 1000 cells and testing the resulting vegetation for sporulation, an abundance of spores being found in every case. The experiments also showed that the intermediate, temporarily asporogenic forms appeared as soon as the treatment commenced; and since they were never found in the original material, their origin must therefore be attributed to the treatment applied. Finally, the variation in question is a universal phenomenon, appearing in all cases when the cells are subjected to the treatment described. The results obtained, both from the analysis of the original material and that of the various stages of the treatment, indicate clearly that the variation produced by the treatment is due to transformation or modification. It has been urged against this view that, since all the cells are of equal value, they must all undergo transformation at the same time if the process is really one of modification. This, however, is incorrect, since the cells are far from being of equal value, their condition at the moment of commencing the treatment differing considerably in respect of age, nutrition, &c., and therefore the transformation cannot proceed simultaneously with all the individuals present.

A highly characteristic feature, which was also tested experimentally by Hansen, is that, even when the initial material consists of a single vegetative cell or spore, the following three classes: sporogenic cells, temporarily asporogenic cells, and constantly asporogenic cells, appear during the treatment. From the two former classes it is possible to take single cells which in turn produce members of all three classes. This also proves the change is due to modification, inasmuch as these intermediate forms, which revert to the sporogenic form when excluded from the treatment, become constantly asporogenic only after treatment for a considerable time.

With regard to the conditions necessary for the modification, it might be thought that the chemical composition of the nutrient medium, the vibration produced by shaking the flask, the aeration of the medium, and the temperature would constitute influential factors. The experiments, however, showed that neither vibration nor a medium of definite chemical composition is essential; and that aeration is incapable of bringing about the change in the absence of the high temperature. The nutrient liquid, vibration, and aeration exercise an

indirect influence, inasmuch as they more or less facilitate reproduction, but the high temperature forms the most important and absolutely indispensable factor.

In the foregoing experiments Hansen used nutrient liquids for the cultures, but he also tried solid media. In the latter case several species produced constantly asporogenic cells when allowed to remain on wort gelatin at 25° C., or at ordinary temperature, and it may be assumed that chemical factors were here in operation. When grown at 32° and 34° C. on wort-agar gelatin under the same conditions as in the experiments with liquid media, *i.e.*, repeated re-inoculations at short intervals, *Sacch. Pastorianus* produced constantly asporogenic cells, which was, however, not the case when the culture was left undisturbed. Here, again, the high temperature shows itself the modifying factor.

The oldest asporogenic varieties obtained from the different species have now been in existence for more than sixteen years, and have remained constantly asporogenic although repeatedly cultivated under highly divergent conditions.

It is found to be the rule that loss of sporogenic power is accompanied by loss of the capacity for producing film growths. In some species the variety has been found to possess a greater reproductive capacity than the original form, and possibly this applies to all. The asporogenic varieties also exhibit considerable fluctuations in respect of the production of alcohol. Since, as mentioned on p. 92, vol. ii., the film cells of *Saccharomycetes* are able to decompose alcohol into carbon dioxide and water, a power not shared by the sedimental yeast cells (at least so long as the liquid is of sufficient depth), the quantity of alcohol formed in wort fermented by an asporogenic (and therefore filmless) variety does not become appreciably less when left to stand in a flask (*e.g.*, Pasteur flask) precluding evaporation.

In addition to the instance of an accidentally produced constant variation observed with *Saccharomycodes Ludwigii* (p. 191, vol. ii.), mention may be made of Lepeschkin's (I.) observation of the formation of mycelium by *Schizosaccharomyces Pombe* and *Schiz. mellacei*. No particulars are given by this worker respecting the conditions under which this result was obtained, but he states that the phenomenon remained constant during numerous generations and that it was found impossible to secure reversion to the original form of single cells.

§ 275.—Hansen's Experiments with Top and Bottom Yeast.

Special interest attaches to Hansen's (XLV.) latest researches into variations in fermentative habit, namely, the appearance of top fermentation yeast cells in a typical bottom yeast, and *vice versâ*. As mentioned on p. 191, vol. ii., he had previously observed the faculty of certain bottom yeasts for temporarily producing top-fermentation phenomena after storage at a low temperature. In this connection he instituted some very comprehensive researches with *Sacch. turbidans* (= *Sacch. ellips. II.*), a trace of a vigorous young vegetation being transferred to Freudenreich flasks charged with a thin stratum of wort and kept at 0.5° C. At the end of three and five months the cultures were examined, by sowing an average sample in wort contained in test-glasses. In every instance the fermentation phenomena were decidedly those of top fermentation, so that all or most of the cells had acquired a top-fermentation habit. Test-glasses were used because of the necessity for employing a deep layer of wort in making comparative observations on top- and bottom-fermentation phenomena. One test performed with 150 cells showed that not a single bottom-yeast cell was present. In order to solve the problem whether the low temperature had produced modification, the vegetation used for the culture at 0.5° C. was subjected to analysis,

the result being that, of 100 cells, one-half gave top fermentation, the other bottom fermentation. On a series of flasks, charged with a thin stratum of wort, being inoculated with cells from each category and kept for 3–4 months at 0.5° C., it was found that no reproduction occurred in the flasks containing the bottom-fermentation cells, whereas, on the contrary, the top-fermentation cells exhibited decided reproduction. The cultures treated in this way were next grown in test-tubes, with the result that the bottom cells again gave rise to bottom fermentation, and the top cells to top fermentation, thus demonstrating that no modification, but only selection, had been effected by the experiment. When Hansen described *Sacch. turbidans* in 1883, it was a bottom yeast; and the formation of top cells—the cause of which is unknown—occurred spontaneously during the period of storage in the laboratory. In the course of a year the bottom cells and top cells continued to behave as such respectively through a long series of cultures; and 1000 cells isolated from each class all produced the same type of fermentation as that of the class from which they originated.

Experiments with the typical bottom yeast, Johannisberg 2, showed that the cultures not infrequently contain 70 per cent. of top-yeast cells, the isolated cells in this case also retaining their characteristic fermentative habit through a long series of cultures.

Whilst in the cases cited above a transition occurred from bottom fermentation to top fermentation, the converse change seems more difficult to bring about. In this connection Hansen carried on several experiments with *Sacch. validus* (= *S. Past. III.*), which is certainly a typical top yeast, but he only succeeded in obtaining a few bottom cells—not exceeding 3 per cent.—in one of the cultures. The vegetations from these cells retained their character as bottom yeast through a series of generations, extending over two years, and under conditions favourable to the production of top fermentation phenomena.

In this manner the old question whether the top- and bottom-fermentation yeasts are independent forms or not has been settled by the demonstration that bottom cells can be developed from top cells, and *vice versâ*. This essentially modifies our previous conceptions (*see* p. 90, vol. ii.). The two forms into which the species is subdivided may exist for a long time, side by side in the same nutrient medium, until the growth of one of them is favoured by the environment, as was the case in the experiments with *Sacch. turbidans* at 0.5° C., where the top-fermentation form increased at the expense of the bottom-cell form until the latter was entirely suppressed.

In contrast with the asporogenic varieties which, as we have already seen, are modifications produced by the influence of a known external factor, high temperature, the appearance of top-yeast cells in a typical bottom yeast must be relegated to the category of variations to which the name mutation was given by de Vries, and comprising all sudden variations due to unknown causes. In most cases the properties of these mutation varieties are hereditary, as we have already learnt in the case of the temporary variations. Thus, the variations in the cell form, the shape and size of the spores, and also the production of mycelium observed by Lepeschkin, must be classed as mutations. The great difference existing between a transformation and a mutation is that the former is produced gradually, the latter suddenly. Both may form the starting-point of new species or races. Throughout the entire vegetable kingdom only very few instances are known where a new variety, capable of transmitting its newly acquired properties permanently to its offspring, has resulted from a transformation brought about by external influences; in fact Hansen's researches on asporogenic varieties constitute the sole experiment performed in this connection.

§ 276. Practical Results of the Researches on Variation. Occurrence in Brewing Practice.

Before bringing this chapter to a close we will examine the practical bearing that the results of the foregoing investigations have on brewing, and also consider the occurrence of variations of yeast type in practice.

By employing an asporogenic variety of pitching yeast in the brewery, the detection of wild yeasts by spore analysis is simplified. It will be remembered (p. 99, vol. ii.) that this method of examination is based on the fact that, at a certain temperature, sporulation occurs sooner in wild yeasts than in culture yeasts. When, however, an asporogenic yeast is used for pitching, the mere presence of sporogenic *Saccharomyces* cells of any kind will suffice to reveal an extraneous yeast. The fact that an asporogenic yeast will produce just as good beer as the original form has been demonstrated by Hansen, who obtained a good normal beer with an asporogenic variety obtained from Carlsberg bottom yeast No. 2 by the treatment already described. It must not, however, be forgotten that in many instances the behaviour of the asporogenic variety in practice will differ appreciably from that of the original yeast from which it was produced.

Will (XXVIII.) has reported an abnormal fermentation phenomena resulting from the presence of film cells or their descendants in the pitching yeast; and A. Jörgensen (II.) has stated that film cells may produce a disagreeable flavour. The production of films, and therefore also their disturbing influence, may be avoided by the preparation of an asporogenic variety, which, as has already been shown, is incapable of film formation (*see* p. 92, vol. ii.).

The production of varieties with an increased or diminished power of producing alcohol is also of importance in practice. Hansen (XLIII.) carried out experiments on this point, and, by cultivating Carlsberg bottom yeast No. 1 in eight successive cultures at 32° C. without aeration, obtained a variety which gave 1–2 per cent. of alcohol (by volume) less in wort containing 10 per cent. of saccharose than the standard No. 1 yeast, whilst at the same time it clarified the beer better. He also obtained a variety with increased powers of alcohol production by growing the same species, Carlsberg bottom yeast No. 1, for several months on wort gelatin with frequent renewal of the medium, whereas 13 per cent. (by volume) of alcohol was furnished by a vegetation of the same original stock when grown in wort, under equal conditions as regards time and renewal of the cultures; finally, in a wort containing 25 per cent. of saccharose, the variety obtained by growing in wort gelatin produced 13.6 per cent. of alcohol from the same final medium. By cultivating the spores of another culture yeast, *Sacch. cerevisiæ*, on yeast-water gelatin, he also obtained a variety furnishing more alcohol than the original stock yeast, the increased production in this case being 3 per cent. by volume, as compared with that given by the stock yeast grown in wort throughout. According to Hansen the matter is one of selection rather than modification; but nothing more definite can yet be expressed on the point. It may, however, be mentioned that considerable differences in fermentative capacity are exhibited by the individual cells of one and the same species in pure culture in wort, even when grown under identical conditions; and this applies also to their clarifying power.

The variety obtained by Hansen from Carlsberg bottom yeast No. 1, by the method employed for producing asporogenic varieties, was characterised by diminished attenuation and gave a beer of greater palate fulness than the original stock; but it exhibited the defect of being too slow in action.

There is evidently a wide field open for the practical application of varieties

of yeast obtained by treating the original stocks in certain ways on the lines indicated above; and important results are undoubtedly obtainable by continuing these researches. We will now deal briefly with the occurrence of variations in practice, premising that lucid experimental investigations in this connection are still lacking. As already stated, such variations have been observed from the time pure-culture yeast was introduced into practice, but there is little use going into details, since all that is known is based on more or less uncertain observation. Reports from practical sources, on the variation known as degeneration in pitching yeast, will be found in the columns of the technical press for some years past; and we will now merely cite two communications relating to injurious variations. One cause of yeast degeneration is ascribed by HAYDUCK (VI.) to the enrichment of the yeast with nitrogen, and he recommends, as a means of regeneration, that the yeast should be allowed to ferment a solution of saccharose before pitching. In the case of a yeast which suddenly began to clarify badly, SEYFFERT (II.) found that the addition of gypsum to the brewing liquor (well water) restored matters to their normal condition. Sudden disagreeable changes with regard to smell and flavour may also arise in practice, the cause being generally attributed to cultivation at abnormally high temperature, excessive rousing of the wort, &c.; and WILL (XXIX.) states that boiling the wort too long in the steriliser may also influence the activity of the yeast. In short, the yeast may be affected by any unusual conditions in brewing; and in this category should be included the experiments of Biernacki, Effront, Hayduck, Heinzelmann and Schulz, with chemical stimulants (*see* vol. i. p. 84). In addition to Hansen, observations on the variation of pitching yeast in practice have also been published by Delbrück, A. Jörgensen, Kukla and Will.

Proof of the temporary character of the variations caused in practice by the influence of the conditions prevailing there is afforded by the fact that the pure-culture system has not only obtained a solid footing in breweries throughout the world, but is also gaining ground daily in other fermentation industries. Certain culture yeasts are particularly constant, others again show a tendency towards variation. Carlsberg bottom yeast No. 1 belongs to the former class, a pure culture of this yeast having retained its character, apart from temporary fluctuations, for more than five years in the fermentation cylinder of the pure-culture apparatus at the New Carlsberg brewery. Various authors have reported on special constancy in culture yeasts, the researches of Irmisch, A. Jörgensen and P. Lindner being worthy of note in this connection.

As we have seen, the practical application of the pure-culture system consists not merely in the preparation of pure cultures of a given species or race, but also in a selection of the vegetations furnished by individual cells. In this way the introduction of pure cultures in the brewery is accompanied by an attempt at race improvement, the requirements being confined not merely to the preservation, by the race or species, of all its properties that are of value to the brewery, but extending to the selection of individuals exhibiting variations of special value for the brewery in question—that is to say, possessing the good properties in an increased degree and with the undesirable qualities eliminated. Of course these results cannot be more than partially accomplished even in the most favourable circumstances. The race improvement in such cases consists in a repeated selection of the best individuals; but the results of the experiments made by Hansen and others in this direction show that it is impossible to lay down any definite rules, tentative experiments being essential. In some cases disappointment will follow, the results failing to come out as desired, owing to uncontrollable circumstances. The matter is entirely different from the mere production of asporogenic races and the like, where the conditions are known and under control. Finally, it must be borne in mind that when the materials for the

experiments in race improvement are taken from the contents of the fermentation vessel in the brewery, one cannot be certain that any genetic connection exists between the race so taken and the original pitching yeast, for they are not necessarily descended from one and the same ancestor even though exhibiting the same botanical characteristics.

Similar communications on race improvements have also been published in connection with wine yeasts, though again without any definite statement of method; and indeed some of these reports, from practical sources, even fail to mention the species of yeast originally employed.

CHAPTER LV.

CLASSIFICATION OF THE FAMILIES SACCHAROMYCETACEÆ AND SCHIZOSACCHAROMYCETACEÆ.

§ 277.—Introduction. Division of the Family, Saccharomycetaceæ.

The fungi to be classified in the present chapter comprise two families, the *Saccharomycetaceæ* and the *Schizosaccharomycetaceæ*, though they were formerly grouped together as a single family under the former title. According to the principles of classification established for the *Saccharomycetaceæ* by E. C. Hansen (XLIX.) in 1904, however, the *Schizosaccharomycetaceæ* form a separate family, and are therefore dealt with by themselves later on (§ 281).[1]

As already explained on pp. 73, 74, vol. ii., the *Saccharomycetaceæ*, which form the subject of §§ 277–280, belong to the *Ascomycetes* class, of which they constitute the lowest family. On the other hand, the position of the *Schizosaccharomycetaceæ* in the botanical system cannot yet be definitely fixed. They appear to form an intermediate link between the *Ascomycetes* and the *Schizomycetes;* but, for practical reasons, they are ranged in this chapter beside the *Saccharomycetaceæ*.

Before proceeding to a systematic description of the species belonging to the two families in question, we will glance briefly at the earlier attempts at classification, and then state the principles which have been utilised in the present rearrangement.

It is difficult to find in any other department of botany greater confusion than existed in the classification of the *Saccharomycetes*, chiefly on account of the fact that so many workers unacquainted with botany have been engaged in the investigation of fermentative organisms.

The first worker to establish endosporulation as the characteristic feature of the genus *Saccharomyces* was Reess (*see* p. 78, vol. ii.), who described the following seven species of this genus: *Sacch. cerevisiæ*, Meyen (*see* p. 82, vol. ii.); *Sacch. ellipsoideus*, Reess (*see* p. 83, vol. ii.); *Sacch. conglomeratus*, Reess; *Sacch. exiguus*, Reess; *Sacch. Pastorianus*, Reess (*see* p. 84, vol. ii.); *Sacch. mycoderma*, Reess; and *Sacch. apiculatus*, Reess. Of these, however, only one, viz., *Sacch. apiculatus*, has been since identified with certainty. Reess did not act consistently in this matter, since, as he himself pointed out, this species does not produce endospores, and therefore should not have been placed with the *Saccharomycetes*, which he expressly declared to be characterised by endosporulation. He describes this budding fungus (for which *see* chap. lxi.) as consisting of "lemon-shaped cells produced by budding, and provided with short apices at each pole; average width 2–3 μ, length 6–8 μ; sometimes elongated as short filaments. New buds are formed solely at the apices of the parent cells, and usually detach themselves at once, rarely remaining joined, in short lengths or

1 On p. 74, vol. ii., the species belonging to these two families were treated as one family, according to the state of knowledge at the time (1901), and for the same reason only three genera, *Monospora*, *Saccharomyces* and *Schizosaccharomyces* were mentioned. The classification is now amended in the light of recent research, and hence the divergence from the statements made on the pages mentioned.

branching. Ascosporulation not detected with certainty, and assignment to the *Saccharomycetes* consequently doubtful."

With regard to *Sacch. mycoderma*, Reess probably based his description on a mixture of *Mycoderma cerevisiæ* or *M. vini* and a species of *Pichia*, since he expressly states that the species produces spores, which (*see* chap. lx.) the *Mycoderma* do not. The five remaining species mentioned by Reess were characterised almost exclusively from the form of their cells, thus rendering their identification impossible.

Most of his contemporaries followed Reess, except C. O. Harz, who rejected all Reess's species but *Sacch. mycoderma*, on the ground that they were only different forms of beer yeast due to altered nutrition.

Hansen's researches on classification are closely interwoven with his work on the biological and physiological sides, and he proceeded consistently from the outset with the assumption that only such yeasts as produce endospores can belong to the *Saccharomycetes*. This conception, the correctness of which was demonstrated in the course of the investigations, was generally accepted and adopted with but few exceptions, chiefly physicians who followed Schlendrian and called all yeasts *Saccharomyces*, whether they form spores or not. A few other workers also took the same view, Saccardo (II.), for instance, continuing in 1889 to confound *Saccharomyces* and non-*Saccharomyces*, a plan also followed by J. Schroeter in his work on the Cryptogam Flora of Silesia (1893).

The characteristics established as the basis of classification by Hansen, and employed in the present work, may now be briefly described. Among the morphological characteristics he assigns an inferior position to cell form, owing to the extent to which this is affected by external influences, most species exhibiting a large number of cell forms (*see* p. 84, vol. ii.). In fact, it is only under definite conditions of culture that the cell form can be utilised as a characteristic of species. Unfortunately, there are still botanists who call all large rounded cells *Sacch. cerevisiæ*, all small oval ones *Sacch. ellipsoideus*, and all elongated cells *Sacch. Pastorianus*, and thus keep to the same standpoint as Reess. The shape of the spores and the production of films are important generic characteristics, and in some cases of species as well. Physiological characteristics are of great importance in classification, especially the critical temperatures of budding, film production, and sporulation (*see* table opposite p. 98, vol. ii.); and also the behaviour of the species toward different sugars (*see* chaps. lxiv. and lxv.), large quantities of yeast and pure sugars being essential for this purpose. In this instance a macroscopical examination is necessary, microscopical tests not affording sufficient accuracy. The yeast is sown in yeast-water containing 5 to 15 per cent. of the sugar in question, and the production of alcohol is tested for. Finally, Hansen employed as a means of differentiation the macroscopic appearance of the vegetations on different solid nutrient media. His methods differ essentially from those of Reess by being entirely of an experimental character, and it follows therefore that the value of the results for purposes of comparison depends on the experiments being carried out under identical conditions.

Lindner employs the appearance of the giant colonies as a specific characteristic; and Will has also done good work in the study of these forms.

The fermentative habit (as top or bottom yeast) has no longer the same importance as a means of classification that it formerly enjoyed, on account of Hansen's recent investigations in this connection (*see* p. 94, vol. ii.).

Even many of the species put forward as new during the past few years are described in such an imperfect manner that they cannot be included in our classification, the reason in many cases being that the newly discovered species have shown the description of the older ones to be insufficient. An example of this kind is afforded by H. Lindner's so-called *Sacch. hyalosporus*, which is

characterised by the production of bead spores. This peculiarity, however, is shared by several other species, and consequently a more complete description is necessary before the species can be identified. Other workers, again, have named species without describing them; and these we are therefore compelled to omit, confining our list to such species that have been so fully described as to render identification feasible. Unless specific mention is made to the contrary, the description in each case is that furnished by the discoverer of the species. The source of information is quoted in each case.

A few preliminary notes of explanation will facilitate due comprehension of our subjoined *résumé* of the division of the *Saccharomycetaceæ* into genera in accordance with the principles established by Hansen (XLIX.) in 1904. Previous to his researches on the fungi in question, nothing had been done beyond the establishment of the *Saccharomycetes* as a separate genus, the division into species being of an unreliable nature; and Hansen was the first to place these investigations on an experimental basis. Examination of the various species discovered in the course of years then revealed the desirability and possibility of elevating the existing genus *Saccharomyces* to the dignity of a family (*Saccharomycetaceæ*), which Hansen divided into eight genera. In the case of two of these, viz., *Monospora* and *Nematospora*, which are briefly described in § 280, some doubt exists as to whether they really belong to the *Saccharomycetaceæ*. The remaining six genera, which, on the other hand, are recognised as true *Saccharomycetaceæ*, can be separated into two main groups.

The first principal group differs from the second, inasmuch as sowings in nutrient liquids furnish sedimental yeast exclusively at the outset, the production of films occurring only at a much later period, if at all. The film is more or less strongly mucinous, the only exception being *Saccharomyces capsularius*, which gives a film resembling that of *Oidium*. It is probable that more accurate observation will reveal the presence of isolated islands of yeast (*see* p. 88, vol. ii.), even in those species at present considered to lack the power of producing films. The endospores of the species belonging to this first group are globular, oval or reniform, smooth and provided with one or two membranes. The spores germinate either by gemmation or the production of a promycelium. The great majority of the species incite alcoholic fermentation. Hansen divides this group into four genera, one being the newly defined genus *Saccharomyces*, described in § 278, whilst the other three are called *Zygosaccharomyces*, *Saccharomycodes*, and *Saccharomycopsis*, and are dealt with in § 279. It need only be mentioned that the genus *Saccharomycodes* was established for the organism previously known as *Saccharomyces Ludwigii*, and a similar species described by Behrens. In its new form, Hansen's genus *Saccharomyces* comprises a large number of species, and is divided into six sub-groups based on the behaviour of the species towards sugars. The hitherto imperfectly characterised genera *Hansenia* and *Torulaspora* are referred to briefly at the end of § 278. The nomenclature of the species included in the newly defined species *Saccharomyces* has been altered considerably, a number of names, hitherto current in Mycology and also used in nearly all the previous chapters of the present work, having been replaced by new ones. Thus, for example, the organism previously known as *Sacch. cerevisiæ I.*, Hansen, is shortened in the new classification to *Sacch. cerevisiæ*; *Sacch. Pastorianus I.* becomes *Sacch. Pastorianus*; *Sacch. Pastorianus III.* is changed to *Sacch. validus*, and so on. Further particulars on this synonym are given at the beginning of the description of the individual species.

The second principal group of the true *Saccharomycetaceæ* is composed of the genera *Pichia* and *Willia*, and is characterised by the production of a film on the surface of the nutrient solution immediately after the same has been inoculated. Hansen had already discovered a representative of each of these new

genera, and at that time called them *Sacch. membranæfaciens* and *Sacch. anomalus* respectively. Similar species were afterwards discovered and described by other workers, Pichi, for instance, identifying some which may be ranked with *Sacch. membranæfaciens*, whilst Will and his pupils found others of the type of *Sacch. anomalus*. Consequently, Hansen named his new genera *Pichia* and *Willia*, in honour of these workers.

In conclusion we give the following

Analytical Summary of the Genera of the Saccharomycetaceæ Family.

The *Saccharomycetaceæ* exhibit the following general characteristics: Monocellular, sporogenic budding fungi. Typical mycelium is formed only by a few species, but all produce yeast cells abundantly. Each cell is a potential sporogenic cell. The spores are monocellular. The number of spores in each parent cell (Ascus) is usually 1–4, seldom as high as 12.

(1) Spores oval, round, pileate or lemon-shaped, with or without projecting rim, *see* 2.
Spores acicular or spindle-shaped, *see* 7.
The cells form sedimental yeast immediately in saccharine nutrient liquids, films being produced only later (if at all), *see* 3.
(2) The cells produce a film at once on the surface of saccharine nutrient liquids; the film appears dry, owing to included air bubbles, *see* 6.
(3) Spore with single membrane, *see* 4.
Spore with two membranes . . . . . *Saccharomycopsis.*
The cells fuse together . . . . . *Zygosaccharomyces.*
(4) No fusion of the cells occurs, *see* 5.
The spores germinate by ordinary gemmation . . . . *Saccharomyces.*
(5) A promycelium is developed in the germination of the spores, and from this budding proceeds with incomplete separation . . *Saccharomycodes.*
(6) Spores round or hemispherical, or irregular and angular. No fermentation . . . . . *Pichia.*
Spores pileate or lemon-shaped with projecting rim . . . *Willia.*
(7) Spores acicular. Parasitic on water-fleas . . . . . *Monospora.*
Spores spindle-shaped, almost filamentous, with a long flagellum; parasitic on hazel-nuts . . . . . *Nematospora.*

§ 278.—The Genus Saccharomyces, with the Genera Hansenia and Torulaspora.

The cells of the species belonging to the genus *Saccharomyces* (E. C. Hansen) produce simple membrane spores which gemmate by germination. In addition to yeast cells a few of them produce a membrane with well-defined septæ.

The first sub-group of this genus comprises the species capable of fermenting dextrose, saccharose and maltose, but not lactose. It includes the following species:

Saccharomyces cerevisiæ, E. C. Hansen. Synonyms: *Sacch. cerevisiæ I.*, E. C. Hansen (XII., XVI., XXXII. and XLVI.) = *Sacch. cerevisiæ*, E. C. Hansen (XLIX.) = *Sacch. cerevisiæ* (partim), Mayer (I.) = *Torula cerevisiæ* (partim), Turpin (I.) = *Cryptococcus fermentim* (partim), Kützing (I.) = *Hormiscium cerevisiæ* (partim), Bail (III.) = *Sacch. cerevisiæ* (partim), Reess (I.). This species has been drawn by Hansen (XII., XVI., XVII. and XXXII.), also in Figs. 127, 142, 144, and 145 of the present work. The cells of the sedimental yeast are usually large and round; and those of the film vegetation at 6°–15° C. are mostly of the same kind, with but few exceptions. The limits of the budding temperature in wort are 40° C. and 1°–3° C. The dimensions of the spores vary between 2.5 and 6 μ, the number in each cell being usually 1–4, rarely 5. The limits of sporulation temperature on gypsum blocks are 37°–37.5° C., and

9°–11° C., the optimum temperature being 30° C. For the production of films on wort these limits are 33°–34° C. and 6°–7° C. The species generally appears as a powerful top-fermentation beer yeast, and was isolated by Hansen (I.) from the pitching yeast of an Edinburgh brewery. Subsequently the same worker detected it in a London brewery. It is one of the many forms previously grouped under the name *Sacch. cerevisiæ*.

Only a small number of the races and species utilised in the brewing industry have been described in the literature, and even then without systematic names, being generally called after the locality or the owner of the brewery where they were discovered, or again bearing merely the number with which they were labelled in the collection (herbarium) of the investigator. The following six may be cited as examples:

Carlsberg bottom yeast, No. 1, E. C. Hansen. One of Hansen's (XLIV.) drawings is reproduced in Fig. 130. The cells are usually oval or pointed. Spores are produced with the greatest difficulty, being found in very small number even after a considerable time (5–6 days at 25° C.). In the brewery (*see* p. 137, vol. ii.) this yeast gives imperfect clarification, but high attenuation, and the beer is excellent, with good keeping qualities.

Carlsberg bottom yeast, No. 2, E. C. Hansen. The cells, which are illustrated in Fig. 131, after a drawing by Hansen, are more uniform in shape than the preceding species, and also produce spores rather more readily. The beer obtained with this yeast does not keep so well, but clarifies better.

Stock 2, H. Will (XXX.). Will's drawing of this species is reproduced in Fig. 139. The cells are round or oval. The limits of sporulation temperature on gypsum blocks are 31° C. and 11° C., the optimum temperature being 25°–26° C. For the production of films on wort these limits are 28°–31° C. and 7°–10° C. This species is a high-attenuation bottom yeast.

Stock 6, H. Will (XXX.). The cells are round or oval. Limits of sporulation temperature on gypsum blocks, 31° C. and 11° C., optimum 28° C. For the production of films on wort the limits are 25°–31° C. and 7°–10° C. A bottom yeast with medium attenuation.

Stock 7, H. Will (XXX.). Cells round or oval; giant cells of regular occurrence. Limits of sporulation temperature on gypsum blocks, 30° C. and 13° C., optimum 25°–26° C.; for the production of films on wort the limits are 25°–28° C. and 4°–7° C. The species is a low-attenuation bottom yeast.

Stock 93, H. Will (XXX.), is illustrated, from a drawing by Will, in Fig. 137. Cells round or oval. Limits of sporulation temperature on gypsum blocks, 30° C. and 10° C., optimum 28° C. Limits of temperature for the production of films on wort, 30°–31° C. and 4°–7° C. A high-attenuation bottom yeast.

Although imperfectly described from the standpoint of botanical classification, mention may be made of three other beer yeasts, which bulk largely in discussions between fermentation technologists, and in treatises by fermentation physiologists, and are also mentioned frequently in the present Handbook, namely, *Saaz yeast*, *Frohberg yeast*, and *Logos yeast*. The former two were isolated by Lindner (XXXI.) at the Institute for Fermentation Industries, Berlin: one for the pitching yeast used at the municipal brewery in Saaz (Bohemia), the other from the yeast from Frohberg's brewery at Grimma (Saxony). Both have been carefully investigated by Delbrück (IX.), Irmisch (II.), Lindner (XXXI.), Reinke (IV.), and others. Logos yeast was isolated by H. van Laer and Denamur (I.) from the pitching yeast employed at Logos and Co.'s brewery in Rio de Janeiro (Brazil). Its origin is unknown, but was probably the sugar-cane. On chemico-physiological grounds A. Bau (VI.) proposed to divide the old collective name *Sacch. cerevisiæ* into four types: *Sacch. cerevisiæ Frohberg*, top fermentation; *Sacch. cerevisiæ Saaz*, top fermentation; *Sacch. cerevisiæ Saaz*,

bottom fermentation; and *Sacch. cerevisiæ Frohberg*, bottom fermentation. In this manner the names Saaz and Frohberg originally applied to two different species of yeast are used to denote types. Various other top-fermentation beer yeasts have also been described by H. van Laer, A Jörgensen, Greg, &c.

The top yeasts also include the well-known distillery yeasts Race XI. and Race XII. (the latter also cultivated in the manufacture of pressed yeast), both of which were isolated at the Institute of Fermentation Industries, Berlin. Compare p. 84, vol. ii., and HENNEBERG (I.).

Saccharomyces Pastorianus, E. C. Hansen. Synonyms: *Sacch. Pastorianus I.*, E. C. HANSEN (XII., XVI., XXXII., and XLVI.) = *Sacch. Pastorianus*, E. C. HANSEN (XLIX.) = *Sacch. Pastorianus* (partim), REESS (I.). This species was illustrated by HANSEN (XII. and XVI.); *see* Fig. 129. The vegetation in wort consists chiefly of sausage-shaped cells, though round and oval cells are also present. The limits of budding temperature in wort are 34° C. and 0.5° C. The spores measure 1.5–3.5 μ in diameter, their dimensions seldom reaching 5 μ. Most frequently they number 1–4, but occasionally, in very long cells, 5–10. The limits of sporulation temperature on gypsum blocks lie between 29.5°–31.5° C., and 0.5°–4° C. (optimum 27.5° C.); and the same limits in respect of film formation on worts are 26°–28° C. and 3°–5° C. The species is a bottom yeast, and was first discovered in the atmospheric dust in a Copenhagen brewery, and afterwards in damaged beer. It is a dangerous pest in the brewery, being capable of imparting a disagreeable smell and strongly bitter taste to the beer (*see* p. 84, vol. ii.). As a rule it also retards clarification. On the other hand, according to MACH and PORTELE (III.), it produces good wines.

Saccharomyces intermedius, E. C. Hansen. Synonyms: *Sacch. Pastorianus II.*, E. C. HANSEN (XII., XVI., XXXII., and XLVI.) = *Sacch. intermedius*, E. C. HANSEN (XLIX.) = *Sacch. Pastorianus* (partim), REESS (I.). This species has been drawn by HANSEN (XII. and XVI.), and is illustrated in Figs. 133 and 136. The cells are of the same form as those of the preceding species, but rather larger. Sporulation occurs in wort between the limits of 40° and 0.5° C., and the spores generally measure 2–5 μ, less frequently 4–5 μ. The limits of sporulation temperature on gypsum blocks are 27°–29° C. and 0.5°–4° C., the optimum being 25° C. In the case of film formation on wort, these limits are 26°–28° C. and 3°–5° C. The cells of the young film, at 13°–15° C., differ from the corresponding cells of the next species in being round or oval, whereas under the same conditions many of the cells of *Sacch. validus* are sausage-shaped. At the end of sixteen days the streak cultures of this species on yeast-water gelatin at 15° C. exhibit smooth edges, in which respect again they differ from *Sacch. validus*. The species is a weak top yeast, and was discovered in the air of a brewery in Copenhagen.

Saccharomyces validus, E. C. Hansen. Synonyms: *Sacch. Pastorianus III.*, E. C. HANSEN (XII., XVI., XXXII., and XLVI.) = *Sacch. validus*, E. C. HANSEN (XLIX) = *Sacch. Pastorianus* (partim), REESS (I.). The cells have been illustrated by HANSEN (XII. and XVI.), and also in Figs. 134 and 135. The cells grown in wort have the same shape as the two foregoing species, and their limits of budding temperature in that medium are 39°–40° C. and 0.5° C. The spores measure 2–4 μ in diameter, rarely 3.5–4 μ. Limits of sporulation temperature on gypsum blocks, 27°–29° C. and 4°–8.5° C.; optimum, 25° C. Temperature limits of film formation on wort, 26°–28° C. and 3°–5° C. The cells of the young film grown at 13°–15° C. differ from the corresponding cells of *Sacch. intermedius*, inasmuch as many of them are very long and sausage-shaped; those of the last-named species being, on the other hand, frequently round or oval. The streak cultures on yeast-water gelatin at 15° C. differ, at the end of sixteen

days, from those of the preceding species in being decidedly bearded at the edges. The species is usually a top yeast, and is injurious to beer, in which it produces yeasty haze (*see* p. 89, vol. ii.). Under certain conditions, however, a small addition of this species to the pitching yeast may clarify opalescent beer, probably by eliminating, in secondary fermentation, the substances causing the opalescence. The species was discovered in bottom-fermentation Copenhagen beer suffering from yeasty haze.

Saccharomyces ellipsoideus, E. C. Hansen. Synonyms: *Sacch. ellipsoideus I.*, E. C. Hansen (XII., XVI., XXXII., and XLVI.) = *Sacch. ellipsoideus*, E. C. Hansen (IX.) = *Sacch. ellipsoideus* (partim), Reess (I.) The species has been illustrated by Hansen (XII. and XVI.), and also in Figs. 128 and 132. The cells are ellipsoidal, though they may also be sausage-shaped. The limits of budding temperature in wort are 40°–41° C. and 0.5° C. The spores are 3–4 μ, seldom 3.5–4 μ in diameter. Limits of sporulation temperature on gypsum blocks, 30.5°–32.5° C. and 4°–7.5° C.; optimum, 25° C. Limits of film-formation temperature, 33° 34° C. and 6°–7° C. The cells of the young film, grown at 13°–15° C. differ from those of *Sacch. turbidans* (which are round and oval) by consisting largely of long, sausage-shaped forms. At the end of eleven to fourteen days the streak cultures on wort gelatin at 25° C. exhibit a peculiar reticulated structure, differentiating them from the preceding species and *Sacch. turbidans*. This species is generally a bottom yeast. It was discovered on the surface of ripe grapes in the Vosges district, and is one of the numerous species that play an active part in the fermentation of wine.

A number of wine and fruit-wine yeasts allied to *Sacch. ellipsoideus* have been isolated and described by Aderhold, Hotter, Kayser, Lindner, Marx, Müller-Thurgau, Nastjukow, Osterwalder, Seifert, Wortmann, and others. One of the best known species is:

Johannisberg II., Wortmann (XVI.), which has been drawn by Aderhold (I.). According to this observer, it is distinguished by copious sporulation, 99–100 per cent. of the cells producing spores on gypsum blocks. Hansen (XXXII.) gives the limits of budding temperature in wort as 37°–38° C. and 0.5° C., and those of sporulation temperature on gypsum blocks as 33°–34.5° C. and 2°–3° C. The species is usually a bottom yeast.

Saccharomyces turbidans, E. C. Hansen. Synonyms: *Sacch. ellipsoideus II.*, E. C. Hansen (XII., XVI., XXXII., and XLVI.), *Sacch. turbidans*, E. C. Hansen (XLIX.) = *Sacch. ellipsoideus* (partim), Reess (I.). This species has been drawn by Hansen (XII. and XVI.). The cell form is generally similar to that of the preceding species. Limits of budding temperature in wort, 40° C. and 0.5° C. The spores are 2–5 μ, seldom 4–5 μ in diameter. Limits of sporulation temperature on gypsum blocks, 33°–35° C. and 4°–8° C.; optimum 29° C. Limits of film formation temperature 36°–38° C. and 3°–5° C. The cells of the young film grown at 13°–15° C. differ from those of *Sacch. ellipsoideus* in being chiefly round and oval. The species occurs as both top and bottom yeast, and is an injurious organism causing yeasty haze in bottom-fermentation breweries. It was discovered, with *Sacch. validus*, in beers affected with yeasty haze (*see* p. 84, vol. ii.).

Saccharomyces Willianus, Saccardo. Synonyms: *Saccharomyces I.* of Will, Bay (II.) = *Sacch. Willianus*, Saccardo (II.). The species was first described and drawn by Will (VIII.), but merely as "yeast No. 11." The cells are ovoid, and the spores measure 1.5–5 μ in diameter, usually 3.5 μ. Not more than 4 spores have been discovered in a cell. Limits of sporulation temperature on gypsum blocks, 39°–41° C. and 4°–9° C.; optimum, 34° C. Limits of film formation temperature on wort, 39°–41° C. and 4° C. The species produces disagreeable flavour and haze in beer.

Saccharomyces Bayanus, Saccardo. Synonyms: *Saccharomyces II.* of Will, Bay (II.). *Sacch. Bayanus*, Saccardo (II.). This species was first described by Will (VIII.), but merely as a "yeast causing beer haze." The cell form is pointed ovoid, turbinate or spindle-shaped, 7–11 μ in length and 5–6 μ in breadth. In old films the length of the cells reaches 30 μ and the breadth 2–4 μ. From two to four spores are produced, their dimensions being 2–4 μ. The limits of sporulation temperature on gypsum blocks are 30°–32° C. and 0.5°–3° C., the optimum being 23.5°–24° C. This species produces both haze and a sweetish metallic and disagreeably aromatic taste in beer, as well as an unpleasant bitter, astringent after-taste. At the same time the beer acquires a peculiar aromatic smell, like rotten fruit.

Saccharomyces ilicis, Grönlund, has been drawn by Grönlund (I.). The cells are mostly globular. The limits of sporulation temperature on gypsum blocks are 36°–38° C. and 8°–9.5° C., the optimum being 32° C. The streak cultures on wort gelatin have a mealy appearance. The species was discovered on the fruit of *Ilex aquifolium*, and is a bottom yeast, producing 2.78 per cent. of alcohol (by volume) in wort, to which it imparts a disagreeable bitter taste.

Saccharomyces aquifolii, Grönlund (II.), forms cells analogous to those of *Sacch. ilicis*. The limits of sporulation temperature on gypsum blocks are 27.5°–31° C. and 8°–10.5° C.; optimum 27° C. The streak cultures on wort-gelatin have a shiny appearance. The species is a top yeast, and probably a culture yeast. It produces 3.71 per cent. of alcohol in wort, and imparts a sweetish flavour, with bitter after-taste, to the beer. The fruit of *Ilex aquifolium* is the natural habitat.

Saccharomyces Vordermanii, Went and Prinsen Geerligs. Drawings of this species have been made by Went and Prinsen Geerligs (I.). The cells are rounded, pear- or onion-shaped, angular or elongated forms being found occasionally. The number of spores is usually four. No film is produced, but only a yeast ring in old cultures. The species is said to produce 9–10 per cent. of alcohol, and was discovered in the "Ragi" employed in the manufacture of Javanese arrack (*see* p. 67, vol. ii.), the product obtained being of very fine quality, devoid of fusel oil.

Saccharomyces pyriformis, Marshall Ward, has been drawn by Ward (II.), and is illustrated in Fig. 97. The cells are generally ellipsoidal or oval, occasionally globular, and measure 5–9 μ in diameter. Four spores are usually produced in a cell, the time of formation on gypsum blocks at 25° C. being twenty-four hours. A film composed of pear-shaped cells, with interspersed sausage-shaped cells, is formed in three weeks on nutrient solutions. The limits of budding temperature are 35° C. and 10° C. The species is a bottom yeast and was discovered in England, in ginger-beer (*see* vol. i. p. 196).

Saccharomyces mali, Risler, Kayser (I.), was drawn by this last-named worker. The cells are generally globular and measure 4–6 μ. The sedimental yeast is very firm. This species does not produce film. The spores develop in ninety-six hours at 15° C. The species is a bottom yeast, found in cider.

Saccharomyces Saké, Yabe (I.), was first described by Kozai (III.) without being named. The cells are generally globular and 6–12 μ in diameter. Giant cells are present in old cultures. Spores are developed on gypsum blocks, in thirty-six hours at 41° C., fourteen hours at 30°–32° C., and fifteen days at 3°–4° C. The number of spores in each cell rarely exceeds 1–3. This species was discovered by Kozai on Koji, and has been successfully employed, as a pure culture, in the peparation of "Saké."

The second sub-group comprises such species as ferment dextrose and saccharose, but not maltose and lactose. It includes:

Saccharomyces Marxianus, E. C. Hansen (XLVI., XLIV., and XXXII.),

which has been illustrated by Hansen (XLIV.). The vegetative cells of this species are small, oval, or ovoid, or else elongated and sausage-shaped, frequently assembling in colonies. Mycelial colonies are formed when the cultures have stood for some time in wort. The limits of budding temperature in wort are 46°–47° C. and 0.5° C. After about three months, wort cultures develop a tender film, composed partly of short sausage shaped cells and partly of oval forms. On solid media the species forms a mycelium resembling that of *Monilia candida* in structure. The spores are more or less reniform, occasionally round or oval, and most frequently about 3–5 μ in length. According to Klöcker (I.), the limits of sporulation temperature on gypsum blocks are 32°–34° C., and 4°–8° C., optimum 22°–25° C. Hansen states that only 1–1.3 per cent. of alcohol (by volume) is produced after prolonged sojourn in wort. In a solution of 15 per cent. of saccharose in yeast water, 3.75 per cent. (by vol.) of alcohol were formed in eighteen days at 25° C., and 7 per cent. after thirty-eight days. In yeast water containing 10 and 15 per cent. respectively of dextrose 6.5 and 8 per cent. of alcohol were produced in one month. The species was discovered on grapes by Marx.

Saccharomyces exiguus, E. C. Hansen (XLVI.) Synonym: *Sacch. exiguus* (partim), Reess (I.) This species forms cells similar to those of the last named, but differs therefrom in not forming mycelial colonies in wort, or a mycelium on gelatin. Sporulation is very scanty, and only a mere suggestion of a film is formed even after several months. Up to 6 per cent. (by vol.) of alcohol was formed in yeast water treated with 15 per cent. of saccharose at 25° C., and 8 per cent. of alcohol in a 15 per cent. solution of dextrose at the end of fourteen days. This species has been found repeatedly in the yeast of a pressed yeast manufactory.

Saccharomyces Zopfii, Artari (I.) has been drawn by the last-named worker. The cells are short, broad ellipsoids or globular, and measure 3–6 μ in diameter, occasionally 8 μ. When the species is grown in a solution of dextrose (*see* p. 154, vol. ii.) containing 5–8 per cent. of ammonium sulphate, septa are developed in the cells. The maximum limit of budding temperature in wort is 33°–34° C., the optimum being 28°–29° C. Spores are readily formed both in fluid and on solid media, the number in each cell being usually two, though occasionally one, three or four are produced. They are globular and measure 1.5–3 μ. The maximum sporulation temperature is about 32° C., and ripe spores are found after twenty-one hours at 29° C. The vegetative cells are stated to withstand 130° C. dry heat and 66°–67° C. moist heat for half an hour. The species was discovered in sugar juice at a sugar works in Saxony.

Saccharomyces Bailii, P. Lindner (XIV.) was drawn by the latter worker. The cells are large, of somewhat elongated shape and with tough membrane, and old cultures exhibit amœba-like cells of irregular form. The spores are highly refractive. Film formation does not occur on nutrient solutions, and only occasionally are small islands of yeast found thereon. The streak cultures on wort gelatin are greyish white and lustrous; and the same colour and appearance are exhibited by the giant colonies which develop slowly on the same medium. No liquefaction of the gelatin occurs. The species was isolated from Dantzig "Jopen" beer (*see* p. 229, vol. ii.).

Saccharomyces Joergensenii, Laschié (I.), was drawn by the last-named worker. The cells are round or oval, measuring 2.5–5.5 μ, and united in short chains, the spores globular, 1–2.5 μ thick and highly refractive, two to three being usually present in a cell, but rarely four. No development of film has been observed, but only a slight yeast ring, composed of round and oval cells. The limits of sporulation temperature on gypsum blocks, after cultivation in dextrose yeast water, are 26°–30° C. and 8°–12° C., with 25° C. as the optimum temperature.

The species was discovered in American "Temperance beer," and when used in wort of the gravity 10.19 per cent. Ball., produces 0.89 per cent. (by weight) of alcohol.

The third sub-group comprises the species which ferment dextrose and maltose, but not saccharose and lactose as well. They are:

Saccharomyces Rouxii, Boutroux (IX.), which was drawn by Boutroux. The cells are round or oval, unite in chains, are very regular and measure 4–5 μ in diameter. No film is developed, but only a few yeast islands here and there. The number of spores in a cell is one, two or three, and they are also formed in the cells on the surface of the medium. The volume of alcohol produced does not exceed 5.3 per cent. even in presence of an excess of dextrose. The species is apparently identical with that mentioned by Roux (II.) and found in dextrose. Boutroux discovered it in fermenting fruit juices. Though imperfectly described, the species is mentioned here on account of its interesting behaviour toward sugars.

Saccharomyces Soja, Saito (I.). This species has not yet been fully described, but the deficiency will be repaired shortly. It is distinguished by the circumstance that invertase is formed within the cells, though no fermentation of saccharose occurs. Lævulose, galactose and mannose are attacked, but not raffinose, inulin or di-methyl glucoside. The species was discovered in "Moromi," the mash employed in the preparation of Soja sauce (*see* chap. lvii.).

The species of the fourth sub-group, which ferment dextrose, but not saccharose, maltose or lactose as well, are two in number.

Saccharomyces mali, Duclaux, Kayser (III.), which was drawn by the last-named. The cells are 6–12 μ long and 4–8 μ wide, and form a loose sedimental deposit. A film is produced. Spores make their appearance at the end of eighty-four hours at 15° C. This species is a top yeast, and was discovered in cider, to which it imparts a fine bouquet.

Saccharomyces flava lactis, Krueger (I.). The cells are small, ellipsoidal, about 3.8–4 μ in diameter, and united in chains. The colonies on gelatin are yellow in colour, and rapidly liquefy the substratum, which they cover with a yellow film. The same appearance is also observed in the sowings on milk and on solutions of lactose. The yellow colouring-matter is formed only in presence of air. The species was discovered in butter, to which it had imparted an abnormal yellow colour and a highly disagreeable smell like stale urine. It is included here on account of the remarkable production of colouring-matter, although only imperfectly described at present.

The species of the fifth sub-group are characterised by their power of fermenting lactose. Hence they belong to the organisms which excite alcoholic fermentation in milk (*see* vol. i. p. 66) and play an important part in the preparation of Kefyr, Koumiss, Mazun, &c. They are but few in number. A species of budding fungus discovered in milk by Grotenfelt (III.) was named by him *Sacch. acidi lactici* (not *S. lactis acidi* as is frequently, but erroneously, written). In respect of this species, and of another previously described by Duclaux (XIV.) and named *Sacch. lactis*, he says that both sporulate on potatoes. Kayser afterwards showed that Duclaux's species cannot produce spores and is therefore a torula; consequently it is also highly probable that Grotenfelt's species is not a *Saccharomyces*. A number of other species also described as *Saccharomyces* are really torulæ (*see* chap. lix.). On the other hand, the following species must be classed as true *Saccharomycetes:* a *Saccharomyces* capable of fermenting lactose, discovered by E. von Freudenreich and O. Jensen (II.) in Emmenthal cheese; two species afterwards isolated from butter by O. Jensen (II.), and one found by Mazé (I.) in cheese. None of them has received a systematic name, and the descriptions are imperfect.

The only species of which a complete description is available and to which a systematic name has been given is the following:

Saccharomyces fragilis, JÖRGENSEN, which has been drawn by that worker (XIII.). The cells are small, oval, and elongated. The spheroidal spores are produced both in fermenting liquids on gelatin, and in gypsum-block cultures, appearing in the latter case after twenty hours at 25° C., and in forty hours at 15° C. Grown in 10 per cent. lactose yeast water at room temperature, the species produces 1 per cent. (by weight) of alcohol in eight days, and 4 per cent. in four months; whilst in wort of the gravity 11 per cent. Balling, it produces about 1 per cent. of alcohol in ten days at room temperature. The species was isolated from Kefyr.

The sixth sub-group of the *Saccharomycetes* is characterised by lacking the faculty of exciting alcoholic fermentation. The only representative known as yet is:

Saccharomyces Hansenii, ZOPF (XIII.). The cells are globular to ellipsoidal and measure 4–11 μ in diameter. Each cell contains one or more fat globules. The inoculation streaks on wort gelatin form lustrous white colonies; the gelatin is not liquefied. The spores are globular and measure 2–4 μ, and occur singly or in pairs. The species forms oxalic acid in solutions of dextrose, galactose, saccharose, lactose, maltose, dulcitol, glycerol, and mannitol. It was discovered in cotton-seed meal. Owing to the brief description (film formation ?), the position of the species is doubtful.

Closely allied to the genus *Saccharomyces* are the two following genera, *Hansenia* and *Torulaspora*.

In the genus *Hansenia*, P. LINDNER (XXXII.), many of the cells are lemon-shaped, in other respects they exhibit the same characteristics (including sporulation) as the genus *Saccharomyces*. Lindner proposed to apply this generic name to "the *Apiculatus* yeasts" without giving any further indications, on the basis that all the "*Apiculatus* yeasts" produce spores. However, since the species named *Sacch. apiculatus* by Reess is asporogenic, it cannot be classed with this genus. For the present, only that species which is morphologically analogous to *Sacch. apiculatus*, but differs therefrom in being sporogenic, can be included in the genus *Hansenia*. A few species belonging to this genus have been discovered by Beijerinck, Lindner and Röhling, but have not yet been more fully described.

In the genus *Torulaspora*, P. LINDNER (XXII.), the cells are small and globular, with a single large fat globule in each, and resemble the cells of *Torula*. Lindner has not yet enumerated the characteristics of this genus either, except to cite as typical the species *Torulaspora Delbrücki*, LINDNER (XXXII.), formerly described and illustrated by him (XXXI.) under the name *Sacch. Delbrücki*. This species exhibits 1–2 spores in a cell, ferments dextrose and lævulose, and was discovered in English ale.

Although the cell form is the only characteristic as yet specified in connection with the two foregoing genera, they have been included here because of the probability of a sufficient characterisation being established later on. For the present they cannot be differentiated from the genus *Saccharomyces*, the cell form alone being insufficient to serve as a generic characteristic.

§ 279. The Genera Zygosaccharomyces, Saccharomycodes and Saccharomycopsis.

The genus *Zygosaccharomyces*, BARKER (I.), coincides in general with the genus *Saccharomyces*, but differs therefrom in respect of the phenomenon of cell fusion, which precedes sporulation.

Zygosaccharomyces Barkeri, SACCARDO and SYDOW (I.) was first described and

drawn by BARKER (I.), but without being invested by him with a systematic specific name. The cells are oval. The limits of budding temperature on wort agar-agar are 37°–38° C. and 10°–13° C. This species develops merely a yeast ring, but no film. Spores are produced, not only on gypsum blocks, but also on various solid media containing wort, and on damp bread, potatoes, ginger, &c. The limits of sporulation temperature on gypsum blocks are 37°–38° C. and 13° C. The species ferments dextrose, lævulose and saccharose, but not maltose, lactose and dextrin. It was discovered in a vessel containing ginger in Mayer's nutrient solution with saccharose.

Zygosaccharomyces Priorianus, Klöcker, was described provisionally by KLÖCKER (IV.), without being named. The cells in young wort cultures are of various forms, round, oval or elongated, and firmly attached together so that the sedimental yeast forms a coherent mass. The largest cells are produced at 13°–16° C., which temperature is on the whole highly favourable to their development, whereas at higher temperatures, *e.g.*, above 27° C., many of them are very small, and at lower temperatures elongated (sausage-shaped) cells are frequent. Old cultures often exhibit very highly elongated, mycelial cells. The limits of temperature for macroscopical development in wort are 36°–38° C. and 3°–8° C. The colonies in plate cultures on wort-gelatin at room temperature occasionally resemble *Peziza* or lichens. At high temperatures the surface of the colonies is smooth, but at 18° C. and lower it is greatly wrinkled or convolute, and often yellow in colour. Film formation is rare, but well-defined yeast rings are often observed. The spores are round or oval, and generally 2–4 in a cell. At 16°–18° C. they form in large numbers on the surface of the wort gelatin, on sterilised carrot slices, and on gypsum blocks that have been immersed in wort instead of water. In ordinary gypsum-block cultures, on the other hand, spores are produced with difficulty if at all. The limits of sporulation temperature on gypsum blocks in wort, and on slices of carrot, are 27°–28° C. and 3°-9° C. The species ferments dextrose and maltose, but not saccharose and lactose. It was discovered on the bodies of honey-bees, and a similar or identical species has been found on humble bees.

In the genus *Saccharomycodes*, E. C. HANSEN (XLIX.), the spores, which are provided with only a single membrane, germinate into a promycelium, and the new cells, produced from this and the vegetative cells by budding, are incompletely separated, a mycelium with well-defined septa being formed. Up to the present two species are known :

Saccharomycodes Ludwigii, E. C. Hansen. Synonyms : *Ludwig's Saccharomyces*, E. C. HANSEN (XLVII.) = *Saccharomyces Ludwigii*, E. C. HANSEN (XVII., XXXII. and XLIV.) = *Saccharomycodes Ludwigii*, E. C. HANSEN (XLIX.). The species has been illustrated by HANSEN (XVII. and XLIV.), and in Figs. 146 and 150. The cells vary considerably in form, the lemon-shape predominating. The limits of budding temperature in yeast are 37°–38° C. and 1°–3° C. Sporulation occurs not only on gypsum blocks and on gelatin, but also in nutrient liquids, *e.g.*, a 10 per cent. solution of saccharose. The spores are 3–4 μ in diameter. According to NIELSEN (I.), the limits of sporulation temperature on gypsum blocks are 32°–34 C. and 2·5°–7·5° C. Hansen reports that the species ferments dextrose and saccharose, but not maltose. The volume of alcohol produced in dextrose yeast water may attain 10 per cent., but does not exceed 1.2 per cent. in wort. The species was discovered by Hansen and Ludwig in the mucilaginous exudation from oak-trees.

This rare genus also comprises a species discovered and fully described by J. BEHRENS (VIII.), though left unnamed by him. The author therefore proposes to call it

Saccharomycodes Behrensianus, Klöcker. The cells are large, and round or

oval; the spores globular, 4–4.5 μ in diameter, and generally 2–3 in a cell, being formed at the end of twenty-two hours at 18°–20° C. Film formation has not been observed. The giant colonies on 10 per cent. must-gelatin exhibit a highly decorative appearance, the dark central, crater-like hollow being surrounded by very delicate concentric striations. The edges of the colonies are pure white, the older middle part being somewhat darker and of a yellow tinge. These giant colonies show numerous cells containing spores. The species ferments dextrose, lævulose and maltose, but not saccharose, lactose and galactose, and was discovered on hops.

In the genus *Saccharomycopsis*, Schiönning (II.), the spores are bi-membranous. During germination the exosporium opens in a different manner in each of the two known species. In other respects the characteristics, so far as they have been ascertained, approximate most nearly to those of the genus *Saccharomyces*.

Saccharomycopsis guttulatus (Robin). Synonyms: *Cryptococcus guttulatus*, Robin (II.); *Saccharomyces guttulatus*, autt.; *Saccharomyces guttulatus*, Wilhelmi (I.); *Saccharomycopsis guttulatus*, Schiönning (II.). A drawing of this species has been given by Wilhelmi and also in Fig. 148. The following description is chiefly derived from Wilhelmi (I.): Cells ellipsoidal, elongated oval with flattened ends, length 6–16 μ, breadth 2–4 μ, with linear or vortical budding. The optimum budding temperature is 35°–37° C. Nothing is known as to the formation of a film. The spores are of elongated oval form, and 1–4 are present in a cell. In germination, the exosporium bursts, with irregular edges, either at the poles or laterally, and gradually contracts to a small residue of indefinite shape. The species thrives on several artificial nutrient media, *e.g.*, on tartaric glycerin agar-agar with an addition of dextrose. It ferments dextrose and saccharose, and was discovered in the alimentary canal of rabbits, less frequently in that of guinea-pigs and in the excrement of these animals.

Saccharomycopsis capsularis, Schiönning (II.) has been drawn by this worker. The cells are sometimes ovoid, sometimes sausage-shaped; and typical septated mycelia are also observed. The limits of budding temperature in wort are 38.5° C. and about 0.5° C., the optimum being 25°–28° C. On nutrient liquids the species quickly forms a decidedly white, irregular, shaggy film; but on solid media it develops into a more or less irregular, white, shaggy vegetation, which turns chocolate-brown in old cultures on wort-gelatin agar-agar. The spores are generally of oblate spheroidal form, with a maximum diameter of 3.5–8 μ, and usually 4 in a cell. The limits of sporulation temperature on gypsum blocks are 34.5°–35° C. and 5°–8° C., the optimum being 25°–28° C. In germination, the exosporium opens in the form of two valves, generally of unequal size, and often remaining for some time attached together at one point and adhering to the germinating spore. The exosporium is stained pink by concentrated sulphuric acid and several other concentrated mineral acids. The species thrives in wort, yeast water, on wort gelatin, wort-gelatin agar-agar, yeast-water gelatin, rice, and bread. It ferments dextrose, lævulose and maltose, but not saccharose, lactose and raffinose. It was discovered in the soil of a meadow in the Swiss Alps.

§ 280. The Genera Pichia and Willia. The doubtful Genera Monospora and Nematospora.

The two main groups of the true *Saccharomycetaceæ* (p. 202, vol. ii.), comprise the genera *Pichia* and *Willia*, the species of which produce a film on saccharine nutrient liquids immediately. The film has a dry, dull appearance, due to the

inclusion of air bubbles, and exhibit well-defined differences from that produced by the genera described in §§ 278 and 279. The spores are of various shapes, with or without a projecting ledge, and have only a single membrane. Several of the species are characterised by the formation of esters, and a few of them do not excite fermentation.

In the genus *Pichia*, E. C. Hansen (XLIX.), the spores are rounded, hemispherical, or irregular and angular. No fermentation is produced. A strong mycelium is formed. The following eight species (*inter alia*) of this genus are known:

Pichia membranæfaciens, E. C. Hansen. Synonyms: *Saccharomyces membranæfaciens*, E. C. Hansen (XXXII. and XLVI.) = *Pichia membranæfaciens*, E. C. Hansen (XLIX.). The species has been drawn by Seifert (II.). The film consists of sausage-shaped and elongated oval cells, rich in vacuoles. Limits of budding temperature on wort, 35°–36° C. and 0.5° C. The colonies on wort gelatin are dull grey, often with a reddish tinge, and the medium is liquefied very quickly. The spores are rounded or hemispherical, and are produced in large numbers both on gypsum blocks and in the films. According to Neilsen (I.) the limits of sporulation temperature on gypsum blocks are 33°–35° C. and 2.5°–7.5° C., the optimum being 30.5°–31° C. Seifert states that the species continues to grow even in presence of 12.2 per cent. (by vol.) of alcohol. It was discovered by Hansen in a mucinous mass exuding from the damaged roots of an elm; and was also found subsequently in impure well-water by Koehler, and in white wines by A. Jörgensen.

Pichia membranæfaciens II. (Pichi). Synomyms: *Saccharomyces membranæfaciens II.* Pichi (I). The species was drawn by Pichi (II). The cells are 5–7 μ long and 3–5 μ broad, or 10–19 μ long and 3–4.5 μ broad. The spores are often round, or slightly compressed or flattened, and measure 2.5–3 μ in diameter. There are usually 3–4 spores in a cell. The asci in the rugose milk-white film are oval, 6 8 μ in length and 3–5 μ in breadth. Few asci are formed on wort at 22°–25° C. This species was found on the leaves of *Euonymus europæus*.

Pichia membranæfaciens III. (Pichi). Synonym: *Saccharomyces membranæfaciens III.*, Pichi (II). A drawing of the species was given by the last-named worker. The cells are 5–7 μ long and 3–4.6 μ broad, the spores 2.5–3.5 μ in diameter. The asci are globular or oval, contain 2–4 spores, and measure 5–8 μ by 3–5 μ. The film produced on wort at 22°–25° C. is uniform, thin and smooth, and contains a large number of asci. This species was produced in "vin des Côtes."

Pichia californica (Seifert). Synonym: *Saccharomyces membranæfaciens, var. californicus* Seifert (I.) The species was drawn by this worker. The cells are mostly oval, occasionally contain a small highly refractive body, and measure 4–8 μ by 3–5 μ. The films are delicate, white and readily sink to the bottom. The spores are globular, 2–4 in a cell and 2–3 μ in diameter, with homogeneous, highly refractive plasma. Only a few sporogenic cells are found in the films at ordinary room temperature; and sporulation ceases on gypsum blocks at 39°–40° C. and 5°–6° C.; the optimum temperature is 34° C. In wines containing 8 per cent. (by vol.) of alcohol, the maximum temperature at which growth proceeds is 33° C., the minimum being 7°–12° C. and the optimum 28°–30° C.; but in beer wort the limits are wider, the maximum, for instance, being over 39° C. (They were, however, not mentioned by Seifert.) The species, which was discovered in Californian red wine, continues to grow when the volume of alcohol attains 12.2 per cent.

Pichia taurica (Seifert). Synonym: *Saccharomyces membranæfaciens, var. tauricus*, Seifert (I.). In this species, which was drawn by Seifert, the cells

are mostly sausage-shaped, elongated, seldom oval, and measure up to 20 μ in length by 4–6 μ in breadth. The films are delicate, readily sink to the bottom, and when kept at room temperature for a short time exhibit an abundance of sporogenic cells. The spores are oval, 4–6 μ long and 3–4 μ wide, and cease to be produced on gypsum blocks at 34° C. and 4°–6° C. respectively. The optimum sporulation temperature is 27°–30° C. The optimum temperature for growth, in wines containing 8 per cent. (by vol.) of alcohol, is 22° C., the maximum being 28°–30° C. and the minimum 5°–6° C. The species which was discovered in Crimean wine has ceased to grow by the time the volume of alcohol reaches 12.2 per cent.

Pichia tamarindorum (Seifert). Synonym: *Saccharomyces membranæfaciens, var. tamarindorum* Seifert (I.) This worker has made a drawing of the species. The cells are mostly very long, seldom oval or pear-shaped, and often contain a small highly refractive body in the protoplasm. The elongated cells measure up to 26 μ by 2–6 μ, the small oval cells 5–6 μ by 2–3 μ. The films are dense, and of white, dusty appearance, rugose in old cultures. When subjected to vibration, they fall to the bottom as large flakes. The spores are almost hemispherical, about 3 μ high and 4 μ maximum diameter, and they usually contain a small central highly refractive body. In many cases the flat side is slightly arched in the middle, with a small projecting rim. Spores are soon produced in abundance in the films at ordinary room temperature; on gypsum blocks the limits of sporulation temperature are below 34° C. and above 1.5° C., and the optimum temperature is 27°–30° C. Giant colonies on wort gelatin exhibit a peculiar reticulated structure. The species was discovered on tamarind must and a vinous beverage prepared therefrom.

Pichia farinosa (Lindner). Synonyms: *Saccharomyces farinosus*, Lindner (XLIV.) = *Pichia farinosa*, E. C. Hansen (XLIX.) The species was drawn by Lindner (XLIV.). The cells are slender, and old cells in particular are often of angular contour. Spores are abundant in the films, but the latter cease to form at 37° C. The film is bright white in colour, folded like crinkled tissue-paper and looks as though strewn with flour. In old cultures on wort gelatin the medium is liquefied. The species was discovered in Danzig "Jopen" beer (p. 167, vol. ii.), and has also been found by K. Saito (II.) in Japanese Soja sauce.

Pichia Radaisii (Lutz). Synonym: *Saccharomyces Radaisii*, Lutz (I.). The cells of this species are of elongated oval form, 8–8.5 μ long and 3–3.5 μ broad with a membrane 0.8 μ thick. The spores are round, usually four in a cell and measure 1.5 μ in diameter. On gypsum blocks they are produced in twelve hours at 22°–23° C.; and the maximum sporulation temperature is 25°–28° C. The optimum temperature of film formation is 23° C., all development ceasing at 37°–38° C. This species does not liquefy gelatin: and the colonies on that nutrient medium assume a red colour after a short time. *Pichia Radaisii* was discovered in "Tibi," from which a Mexican beverage is prepared.

In the genus *Willia*, E. C. Hansen (XLIX.), the spores are pileate or lemon-shaped, with a projecting rim. Most of the species possess considerable ester-forming powers, but a few lack the capacity of exciting fermentation. The genus comprises the following seven species:

Willia anomala, E. C. Hansen. Synonyms: *Saccharomyces anomalus*, E. C. Hansen (XVII. and XXXII.). *Willia anomala*, E. C. Hansen (XLIX.). The species has been illustrated by Hansen (XVII.), and in Figs. 143 and 147. The microscopic aspect of the cells recalls that of a *Torula*. They are small in size and oval, occasionally sausage-shaped (especially in old cultures). The limits of budding temperature in wort are 37°–38° C. and 0.5°–1° C. At the commencement of fermentation the film is dull grey, the liquid gradually

becoming cloudy. After awhile, sporogenic cells can be detected both in the film and in the sedimental yeast. The cells contain 2–4 spores, which are hemispherical with a projecting rim around the basal surface, so that they present a hat-like appearance. The diameter of the basal surface measures 2–3 μ, irrespective of the rim. According to NIELSEN (I.) the limits of sporulation temperature on gypsum blocks are 32°–34° C. and 2.5°–7.5° C., the optimum being 30° C. A powerful odour of fruit ester is disengaged during fermentation. Nielsen states that the volume of alcohol and ester produced in wort by this species in eleven days is only 0.9 per cent.; and according to Seifert the ester so formed is the ethyl ester of acetic acid. This worker also states that the species decomposes alcohol to water and carbon dioxide, the acetic ester being also consumed eventually. According to Nielsen, *W. anomala* ferments dextrose, but not maltose or lactose, and very little invertase is produced; but other investigations have shown the production of invertase to be decidedly apparent. The species was first discovered by Hansen in an impure Bavarian beer yeast, and it was afterwards found in English beers, on green malt, bran, marshmallow sap and soil, as well as on plums and other fruit. KLÖCKER and SCHIÖNNING (VI.), KOZAI (I.), and SAITO (I.), have found it in the Koji used in the preparation of Saké; and, according to INUI (I.), it is also present in the Koji employed for making "Awamori" in the Loochoo Islands. P. LINDNER (XXXI.) found the same species in the Armenian beverage, Mazun.

Willia anomala I. (Steuber). Synonym: *Saccharomyces anomalus, var. I.*, STEUBER (II.). This species was drawn by its discoverer. The film on wort is initially smooth and chalk-white, but later folded and yellowish. The limits of film-formation temperature are 37°–42° C., and 5°–10° C. The giant colonies on 10 per cent. wort gelatin are yellow in the centre and white, with a silky sheen, at the edge. Giant cells, up to 15 μ, are found in the central portion of the colony, and cells up to 30 μ in length at the edges. The gelatin is liquefied. The spores are pileate, and are produced both in the film on gelatin and on gypsum-block cultures. The limits of sporulation temperature on the latter cultures are 30°–35° C., and 5°–12° C. The species ferments dextrose, lævulose and saccharose, but not maltose, lactose or galactose. It produces acetic esters and acetic acid, and was discovered in water which had been used for washing yeast.

Willia anomala II. (Steuber). Synonym: *Saccharomyces anomalus, var. II.*, STEUBER (II.). A drawing has been made by that worker. The film on wort is smooth and chalk-white at first, afterwards implicate, and turns pink to brownish pink in a short time. The limits of film-formation temperature are 30°–35° C., and 5°–10° C. The giant colonies on wort gelatin soon turn pink to brownish red, and the gelatin is liquefied. An abundant formation of pileate spores is observed, and the limits of temperature for this phenomenon on gypsum blocks are 30°–35° C., and 5°–15° C. With regard to the behaviour of the species toward sugars, Steuber says: "A 10 per cent. solution of saccharose is inverted and fermented completely, though slowly, only 0.45 per cent. of alcohol is produced in a 10 per cent. solution of lævulose. It does not ferment dextrose, lactose, galactose or maltose, merely traces of alcohol (if any) being produced in those solutions. No acetic ether is formed." There appears to be some error in this statement, for if a yeast cannot ferment dextrose, it is also incapable of completely fermenting an inverted saccharose solution.

Willia anomala III. (Steuber). Synonym: *Saccharomyces anomalus, var. III.*, STEUBER (II.) Drawn by this worker. The film is white at first, yellowish afterwards. The limits of film-formation temperature are 30°–35° C., and 5°–15° C. The giant colonies on wort gelatin are white and irregular, and liquefaction of the medium is produced. Limits of sporulation temperature on

gypsum blocks, 30°–35° C., and 5°–15° C. "In a 10 per cent. solution of lævulose, 0.4 per cent. of alcohol is produced in four weeks. The species does not ferment dextrose, saccharose, lactose, galactose or maltose; nor is any acetic ether formed."

Willia belgica (Lindner). Synonym: *Saccharomyces anomalus, var. belgicus*, Lindner (XXXI.). The species was drawn by the last-named worker. It grows on wort as a creamy, punctated film; the cells are comparatively small, thin-walled and poor in contents. The pileate spores are mostly developed in such abundance that little but the sharp lines of the projecting rims can be seen. The species does not ferment any known sugar, nor does it produce fruit esters. It was discovered in Belgian beer.

Willia Saturnus (Klöcker). Synonym: *Saccharomyces Saturnus*, Klöcker (V.). Drawn by the last-named. The film is white and rugose, the cells round or oval, seldom elongated, usually 4–6 μ long. The limits of budding temperature on wort are 35°–37° C. and 2°–4° C. The spores are more or less decidedly lemon-shaped, about 3 μ in length, with a projecting peripheral ledge extending from tip to tip, and containing a small central refractive globular body. The limits of sporulation temperature on gypsum blocks are 28°–31.5° C. and 4°–7° C., the optimum being about 25° C. This species ferments dextrose, lævulose, raffinose, and saccharose (the latter after inversion), but not maltose, lactose, or arabinose. An ester (acetic ester?) is produced during fermentation. The organism was discovered in samples of soil from the Himalayas, and the same or an allied species has been repeatedly found in Danish and Italian soils.

Although, as already mentioned on p. 201, vol. ii., doubt exists as to whether the genera *Monospora* and *Nematospora* really belong to the family *Saccharomycetaceæ*, they will be dealt with in this place.

The genus *Monospora*, Metchnikoff (III.), ought really to be re-named, since this title has already been applied, by Hochstetter, to one of the *Flacourtiaceæ*. In *Monospora*, Metchnikoff, the spore is acicular, and germinates by producing a lateral pro-mycelium, from whence gemmation proceeds. Only a single spore is formed in a cell. The genus contains only one known species, viz., *Monospora cuspidata*, Metchnikoff (III.), which has been drawn by the last-named worker. The cells are an elongated oval. The asci are very long and sausage- or club-shaped, and each ascus produces only a single, acicular spore, pointed at both ends. This species is parasitic in the stomach of the water-flea (*Daphnia*), but since its discovery by Metchnikoff it has not been observed again.

In the genus *Nematospora*, Peglion (III.), the spore is elongated, spindle-shaped, with a long flagellum at one end. Germination proceeds by budding at one or both extremities. Several spores are formed in a cell. Up to the present only one species has been described, namely:

Nematospora Coryli, Peglion (III.), which was drawn by that worker. The cells are elongated, but in old cultures they are round or oval, with a double, lustrous membrane. Budding proceeds from the ends of the cell, as in the case of *Dematium*, but, in nutrient liquids, only a mycelium is formed and no budding occurs. The ascus is sausage-shaped, 65–70 μ long and 6–8 μ broad, and it contains 8 spores, in two bundles of 4 each, disposed along the longitudinal axis. The spores measure 38–40 μ in length, exclusive of the flagellum, which is 35–40 μ long. The thickness of the spores is 2–3 μ. Previous to germination the spores shed the flagellum and become shorter and thicker. The species thrives best, and also sporulates, on sterilised sugar beet, and will also develop on nutrient meat-broth gelatin, but grows very badly in nutrient liquids. It was discovered in hazel-nuts in Italy.

§ 281. The Family Schizosaccharomycetaceæ.

The species of this family are monocellular fungi, which reproduce by fission, and exhibit endosporogenation. The fission of a cell is preceded by the formation of a septum, which at once commences to divide into two lamellæ from the outside. No budding occurs. Each cell may be sporogenic. The spores are monocellular, and 1–8 are formed in the parent cell. At present the family comprises only a single genus, viz.:

Schizosaccharomyces, P. Lindner (XXX.), the generic characteristics of which are also those of the whole family. In some instances the formation of asci is preceded by fusion. All the three species known at present produce spores which are stained blue by a solution of iodine in potassium iodide (*see* p. 107, vol. ii.). The species excite alcoholic fermentation in various sugar solutions. According to Guilliermond the cells never contain glycogen, in which respect they present a contrast to those of the *Saccharomycetaceæ*.

Schizosaccharomyces Pombe, Lindner (XXX.), has been drawn by the last named. The cells are cylindrical, 5–9 μ long and 4–9 μ broad, but the dimensions fluctuate considerably. As a rule the two ends of each cell differ in appearance, the one being rounded, the other surrounded by a sharply defined ring embracing the newly formed membrane, which already assumes a conical shape. Hammer-shaped cells are not infrequently observed. The cells are shorter in exhausted media. With restricted admission of air many of the cells develop into long tubes, containing numerous septa without, however, undergoing separation, and even when the latter occurs, the cells frequently remain attached at one point, as though hinged. According to Guilliermond (II.), the formation of asci is preceded by the fusion of two cells, which may be sister cells, and he also observed instances in which fusion between three cells took place. Sporulation readily appears, even in hanging wort drops, the spores forming sometimes in seven days. Spores are also found in the sedimental yeast at the close of primary fermentation. 1–4 lustrous spores, measuring about 4 μ, are formed in a cell, and these begin to germinate by swelling up to form an ascus, the spore membrane fusing into the new integument without bursting. As soon as the ascus has attained a length about equal to that of an ordinary vegetative cell, it develops a septum and splits into two halves. No formation of film takes place. At high temperatures the fermentation in beer wort is of top-fermentation character. The species ferments dextrose, maltose, and saccharose, and, in addition, lævulose, inulin, dextrin, and raffinose, but not *d*-mannose (in which respect it differs from *Schizos. mellacei*). A boiled mash of malt, potato starch, and saccharose was attenuated from 27.7 per cent. Balling to 1.6 per cent., and then contained 15.5 per cent. (by vol.) of alcohol. The species was discovered in Pombe (African millet beer) by Saare, and was isolated by Zeidler. Lindner states that it has been successfully used in a distillery in Argentina.

Schizosaccharomyces octosporus, Beijerinck (XVIII.), has been drawn by Beijerinck (XVIII.), and Schiönning (I.), and is illustrated in Fig. 125. The vegetative cells of this species, grown in beer-wort cultures, are partly cylindrical, partly oval, and measure, according to Schiönning, 4.5–6 μ in breadth and 7–13 μ in length. A yeast ring is formed, as in the case of *Schizos. Pombe*. The asci are of regular oval shape, 14–20.5 μ long by 6–10.5 μ broad, and usually contain 8 spores, though 4 are often found, but rarely 2–7. Sporulation occurs both in nutrient liquids and especially on solid media. According to Seiter (I.) spores are formed on gypsum blocks in six to seven hours at 25° C. In this species the formation of the asci proceeds in three different ways: (1) In the manner observed by Schiönning and described on p. 103, vol. ii., namely, the

division of a cell into two daughter cells which fuse together again. (2) By the fusion of two cells not derived from the same parent cell (*Guilliermond*). (3) Without the occurrence of any cell fusion at all (*Guilliermond*). The species does not produce any film, but only a slight yeast ring, and rapidly liquefies wort gelatin. It ferments dextrose, maltose, and, lævulose, and, according to Lindner, dextrin, raffinose, and *d*-mannose as well, but it is incapable of fermenting saccharose. Schiönning states that it gives rise to bottom-fermentation phenomena in a slight degree in wort (gravity 14 per cent. Ball.), and at the end of three weeks at 25° C., produces 4.6 per cent. (by vol.) of alcohol, increasing in five months to 6.56 per cent. It was discovered by Beijerinck on currants, and by Schiönning on raisins.

Schizosaccharomyces mellacei (A. Jörgensen). Synonym: *Saccharomyces mellacei*, A. Jörgensen (XIII.). The species was drawn by this worker. The cells are 8–12 μ long and 4–6 μ broad, and resemble those of *Schizos. octosporus* and *Schizos. Pombe*. Peculiar, oddly formed cells appear in old cultures. According to Guilliermond (II.), the ascus is formed by the fusion of two cells, frequently sister cells, though in a variety of the species he observed the ascus seemed to be formed without any previous cell fusion. The spores measure about 4 μ in diameter, and are slightly elongated; there are usually 4 in a cell; they are highly refractive. No film is produced, but merely a yeast ring. Lindner states that the species ferments dextrose, maltose, and saccharose, together with lævulose, inulin, dextrin and raffinose. It differs from *Schizos. Pombe* by its greater dimensions, and the property of fermenting *d*-mannose. In beer wort (gravity 10.5 per cent. Ball.) it gives rise to top-fermentation phenomena, and produces 2.5 per cent. (by weight) of alcohol. An agreeable aroma is disengaged during fermentation. The species was discovered by P. Greig in cane-sugar molasses used in Jamaica for the production of rum.

SECTION XV.

MORPHOLOGY, PHYSIOLOGY AND CLASSIFICATION OF CERTAIN TECHNICALLY IMPORTANT HIGHER ASCOMYCETES AND ALLIED FORMS.

CHAPTER LVI.

MORPHOLOGY AND SUBDIVISION OF THE FAMILY ASPERGILLACEÆ.

By Prof. Dr. CARL WEHMER.

§ 282. Systematic Position and Classification of the Aspergillaceæ.

The systematic position (as *Ascomycetes*) of the *Aspergillaceæ*—a family rendered chemically interesting and technically important by many of its representatives —has already been defined on p. 73 of the present volume. Consequently we have now chiefly to deal briefly with its subdivision.

The *Aspergillaceæ*, which stand next to the *Gymnoascææ*, but are distinguished from these latter by the possession of carpoasci surrounded by an integument, differ from the majority of *Carpoascææ* (*Pyrenomycetes*, *Discomycetes*) by the irregular distribution of the asci in the carpoascus, and, on the other hand, from the otherwise similar truffle-like fungi (*Elaphomycetes* and *Terfeziaceæ*)—which mostly produce large subterranean fruit—by the smallness of their carpoasci. The asci in these fruits —which for the most part do not burst open in ripening, but either remain closed or else break up irregularly—develop 2–8 monocellular spores. According to the character of the carpoasci, and more especially in accordance with the structure of the highly divergent conidiophores—which often predominate or are present exclusively—Ed. Fischer (II.) has latterly divided the family into twelve genera. Schröter (I.) in 1893 counted only four, whilst G. Winter (IV.) in 1887 allocated the genera of this family to the sub-order of *Perisporiaceæ* (*see* p. 73, vol. ii.).

Subjoined is a

Synopsis of the Genera of Aspergillaceæ according to Ed. Fischer (II.).

(*A*) Carpoasci with mostly pseudoparenchymatic Peridium, uniformly filled with Asci.

(*a*) Carpoascus cervicate or with protrusive papillus . . . . . *Microascus.*
(*b*) Carpoascus acervicate :
  (*α*) Peridium with spirally coiled appendices . . . . . *Magnusia.*
  (*β*) Peridium with straight hairs or a shaggy coat
    1 Peridium of more or less carbonaceous nature . . . . *Cephalosthea.*
    2 Peridium membraneous . . . . . *Aphanoascus.*

(γ) Peridium devoid of appendix :

1. No conidia, merely breeding-cells . . . . . *Anixiopsis.*
2. Conidia formed in chains directly on the mycelium, with endogenous spores as secondary organs of fructification . . *Thielavia.*
3. Conidia on conidiophores with terminal swelling, studded with numerous simple or branched sterigmata, in chains . . *Aspergillus.*
4. Conidia on sympodial branched conidiophores in chains . . *Allescheria.*
5. Conidia on branched conidiophores . . . . . *Penicillium.*

(*B*) Carpoasci rounded or pear-shaped, with dense, stratiform peridium. Asci mingled with capillitium threads. The carpoasci undergo dehiscence by opening at the crown or decay of the upper part of the peridium.

(*a*) Asci with dentate projections, spores with equatorial fillet . . *Emericella.*
(*b*) Asci ellipsoidal, with blunt projection at the crown, spores with fine, hairy spines . . . . . *Amylocarpus.*
(*c*) Carpoascus nodular, stalked, with thick peridium, proceeding from sterile veins separating the asciferous network inside the carpoascus . . . . . *Penicillopsis.*

Not to be confounded with this "natural" family of *Aspergillaceæ* is the group established under that name, as a subdivision of the *Mucedineæ* (*see* p. 6, vol. ii.), solely on the basis of the structure of the conidiophores. To this group applies the following synopsis, differing somewhat from that of Lindau (II.).

(*A*) Conidiophores invariably distended at the apex, in the form of a bladder or globule :

1. Conidiophore unbranched :
   (*a*) Chains of conidia formed merely at the apex of the sterigma :
      (α) Simple unbranched sterigmata . . . . . *Aspergillus Citromyces* (*see* below).
      (β) Branched sterigmata, with occasional simple forms . *Sterigmatocystis.*
   (*b*) Chains of conidia, forming at the apex and below the septum . *Dimargiris.*
2. Conidiophores with dichotomous branchings . . . . . *Dispira.*

(*B*) Conidiophores without any (regular) distension at apex :

1. Chains of conidia springing from sterigmata at the apex :
   (*a*) Conidiophores with branches arranged in regular whorls ; conidia barrel-shaped . . . . . *Amblyosporium.*
   (*b*) Conidiophores without regular whorls, simple or branched. Conidia globular or ellipsoidal :
      (α) Conidia without mucinous matrix :
         1. Conidiophores unbranched, with a terminal tuft of sterigmata, and with or without terminal swelling . *Citromyces.*
         2. Conidiophores always more or less regularly branched, without terminal swelling . . . . . *Penicillium.*
      (β) Conidia united to a terminal head by mucinous matrix . *Gliocladium.*
2. Chains of conidia, without sterigmata, formed at the apex of the conidiophore . . . . . *Briarea.*

The number of genera coming under consideration for our purpose is limited to four : *Aspergillus*, *Penicillium*, *Citromyces*, and *Allescheria* (= *Eurotiopsis*), the sole distinguishing characteristic of which consists in the shape of the conidiophores, and not in that of the asci. Indeed, this is still unknown in most of the species now in question. Nevertheless, there does not appear to be sufficient justification for excluding these latter species and treating them separately as "fungi imperfecti," any more than there is for separating the *Mucorineæ* which produce zygospores from those in which zygospores have not yet been observed. (In this family also the spore-carriers in many cases form the sole generic characteristic.) Consequently, for the time being, we will define these three

main genera solely in accordance with the form of the conidiophores, and without reference to the presence or special character of the asci (which would lead to a rearrangement of the grouping), the latter being postponed until more complete knowledge has been gained of the numerous species still outstanding. At present the forms with conidiophores of the *Aspergillus* and *Penicillium* type may be divided into four groups, namely, species with

(*a*) Soft-skinned carpoasci with continuous development (perithecia) : *Aspergillus glaucus, A. fumigatus, Penicillium luteum.*
(*b*) Tough carpoasci with intermittent development (sclerotia): *Penicillium glaucum, Aspergillus nidulans.*
(*c*) Sterile sclerotia, no asci being formed : *Aspergillus flavus, A. ochraceus, A. niger, Penicillium italicum.*
(*d*) Without any organs of the kind : *Aspergillus oryzæ, Penicillium olivaceum,* &c. The majority belong to this class.

Groups (*c*) and (*d*) are only provisional at present, and intermediate forms between the first two are also known to exist (*A. nidulans* approximates to group (*a*)); moreover, there is no concordance between the structure and development of the perithecia and sclerotia of the various species, the differences in some respects being sufficient to necessitate separation. For instance, the carpoascus of *P. luteum* resembles a gymnoascus more than that of *A. glaucus.* Hence the proposal to subdivide the "morphological genus" *Aspergillus* into the genera: *Eurotium* (= (*a*)), *Aspergillus* (= (*b*) and (*c*)) and *Euaspergillus* (= (*d*)), with which would be included the genus *Sterigmatocystis* (*St. nidulans* with carpoasci) —established solely on the basis of conidiophore structure—is unsatisfactory as leaving *Penicillium* out of consideration. Moreover, this proposal does not rest on a proper basis so long as the genus *Penicillium* is left undivided into perithecial, sclerotial and sterile forms; and, finally, the two could be amalgamated by abandoning the conidiophores as the generic characteristic. Contrary to the former disagreement between investigators—compare the works of A. de Bary (VIII.), van Tieghem (IV.), Winter (IV.), and others—there is now, happily, a general desire to include all forms under a uniform name (*Aspergillus*). This has been done by Schröter (I.), with the sole exception of *Sterigmatocystis,* and also *in toto* by E. Fischer (II.), who also included the genus *Eurotium.* At present this is the most commendable attitude to assume, and it must be left to the future to show whether the *Aspergillaceæ* can be—as is desirable—classified from the shape of the fruit alone. The existing defect is probably smaller than that which would be caused by separating the groups characterised by their conidiophores, since it would entail the grouping of divergent conidiophores (*Aspergillus, Penicillium,* &c.) in one and the same genus, and thus reducing the conidiophore to the level of a specific characteristic. Perhaps that may prove to be a way out of the difficulty. In the meantime it is clear that, in these genera, the conidiophores connect a number of forms which differ more or less among themselves in the history of their development.

The genus *Aspergillus* (Mich.), Corda (including *Eurotium*, Link, and *Sterigmatocystis,* Cramer), possesses conidiophores which, for the most part, stand rigidly upright and tougher than the vegetative hyphæ, 0.2–4 mm. in length (seldom more), carry a terminal swelling, and are usually unbranched and aseptate, *i.e.*, monocellular. The conidial chains spring simultaneously from simple or branched sterigmata, as radial or tufted projections from the swelling. Up to the present, conidiophores are known to exist in only a few species, on which they appear as small, coloured, globular capsules or nodules with a delicate single integument, or tougher, stratified skin either with or without a separate husk; the asci (containing 8 spores) either develop at once or after a short period of repose, or again remain sterile a long time. The fruit develops either from one or two

special hyphæ, or by the fusion of a number of ordinary hyphæ. The number of species is uncertain, over 100 having been set up, but barely 20 fully described. The conidial herbage is green, yellow, reddish brown, blackish brown or white.

The genus *Penicillium*, Link, produces conidiophores, which are delicate, barely distinguishable from the ordinary hyphæ, always less than 1 mm. in height, with septate stalk, polycellular, branched alternately or in whorls near the apex, and without terminal swelling. The conidial chains are produced on simple successive sterigmata which, in most cases, form tufts on the ends of the branches. The conidiophores, where such are known to exist, resemble those of *Aspergillus*, being delicate or tough, with or without a cortical envelope, developing continuously or intermittently, or remaining sterile for a time according to the species, and are usually formed by the fusion of two similar hyphæ (*P. glaucum*, Brefeld). The number of species is still uncertain, about 100 having been set up, but only about 12 properly described. The conidia form a herbage, generally green in colour, more rarely white, red, brownish yellow or brown.

The genus *Citromyces*, Wehmer, has delicate conidiophores, like those of *Penicillium*, but unbranched, carrying a tuft of sterigmata with more or less developed terminal swelling, sparsely septated or not at all. The chains of conidia are invariably arranged as projections formed in succession on the swelling or apex of the stalk of the sterigmata, which may be single, tufted or whorled. The herbage is green. Asci are unknown. Two species have been more fully investigated.

The genus *Allescheria*, Saccardo and Sydow (= *Eurotiopsis*, Costantin), has sympodial branched conidiophores, from which chains of oval conidia are formed by constriction, which differ appreciably in other respects from those of the foregoing genera. The carposci are globular (perithecia), and the asci contain 8 spores. The herbage is white to reddish or red. Up to now only a single (rare) species is known.

§ 283. The Genus Aspergillus.

In this genus we include all the mould fungi possessing the characteristic *Aspergillus* conidiophore (with globular terminal swelling developing sterigmata), and do not set *Sterigmatocystis* (with branched sterigmata) or *Eurotium* (forming perithecia) apart as separate genera.

The genus, characterised by the shape of the conidiophores, comprises a considerable number of species that are not always easily differentiated, and for whose identification the morphological details of that organ are of importance. In fact these details alone are sufficient to characterise the species in many cases; and this is the point with which we are now concerned, not with the investigation of the obscure conditions of relationship. With regard to the genus *Aspergillus*, the reader is also referred to the works of Wilhelm (I.), Siebenmann (I.), Tiraboschi (I.), and Wehmer (XVII.), as well as to the recent publications of the French authors, dealing with pathogenic fungi and cited in connection with *A. fumigatus* (p. 234, vol. ii.).

The conidiophore, which, in the mature condition, is far broader and has thicker walls than the vegetative hyphæ, is mostly unbranched and aseptate, springing from a vertical hypha with globular terminal swelling. As a rule, it is clearly separable into stem and globule (*see* Fig. 163), the last-named being covered all over or on the top with a large number of closely set sterigmata of variable length and shape and producing conidia, either direct, or after the formation of secondary sterigmata. The conidia are globular or ellipsoidal, always unicellular, with delicate smooth or finely granular walls, and grow in

FIG. 163.—Conidiophores of Aspergillus.—Heads, globules and sterigmata of *A. Ostianus* (1), *A. glaucus* (2), *A. fumigatus* (3), *A. varians* (4), *A. clavatus* (5), *A. Wentii* (6), *A. sulfureus* (7), *A. nidulans* (8), and *A. candidus* (9). Excretion of granules from stalk and globule in *A. Ostianus* (10). Old sterigmata and globule of *A. candidus* (9, *a–c*). Fragment of globule from *A. giganteus* (high and medium adjustment combined): 11.—Magn. of all the conidiophores approximately equal (about 20–30), except *A. fumigatus* (140) and *A. nidulans* (about 80); of 7*b*, about 270; of 10*a*, 230; of 11, 350. (7 after *Zopf*, 8 after *Eidam*, the rest after *Wehmer*.)

long, wreathed chains, and in addition to covering the heads with a loosely coherent dust (usually coloured), also impart to the herbage of the mould its specific colour (green, blackish brown, yellow-brown, yellow, &c.). The globule, which is not morphologically constant for the species, and may be spherical, oval or elongated, in which latter case it does not exhibit any sharp line of demarcation from the stem, which it also resembles generally in being colourless, tough-skinned, and occasionally very brittle (*A. minimus*). In the microscopical examination of the head (freed from conidia and lightened in the course of preparation) the most important features are the relative length (in comparison with the globule), and more especially the radial (*A. niger*) or upright (*A. fumigatus*) position of the sterigmata. The number of ultimately developing secondary sterigmata (or sterigmata of any order in comparison with the supporting basidia) varies from 2 to 12 according to the species and other circumstances, and these are all considerably more delicate and shorter than the primary forms. The form of the conidia and the character of the membrane (smooth or rough) may vary in the same species (though chiefly through the influence of the medium or of age), and their dimensions sometimes differ considerably (*A. Tokelau*, *A. oryzæ*, *A. flavus*), even in those from the same head, probably as the result of growth subsequent to constriction. In other cases, however, great regularity is observable on these points (*A. niger*, *A. clavatus*), so that in many instances the dimensions afford a reliable diagnosis. In view of the variable dimensions of the conidiophores noted in one and the same culture, apart from differences in nutrition and temperature, the value of accurate microscropical measurements is after all merely relative, though they cannot be entirely dispensed with and are even capable of affording valuable indications when intelligently applied. While scarcely necessary for the mere differentiation of dwarf and normal growths, the measurements ascertained, nevertheless, serve to define more clearly the object examined. The fact that simple and branched sterigmata are found associated in certain species (*A. spurius*, *A. candidus*, *A. ostianus*)—which, therefore, constitute intermediate types—is not altogether favourable to the subdivision of a separate genus, *Sterigmatocystis*.

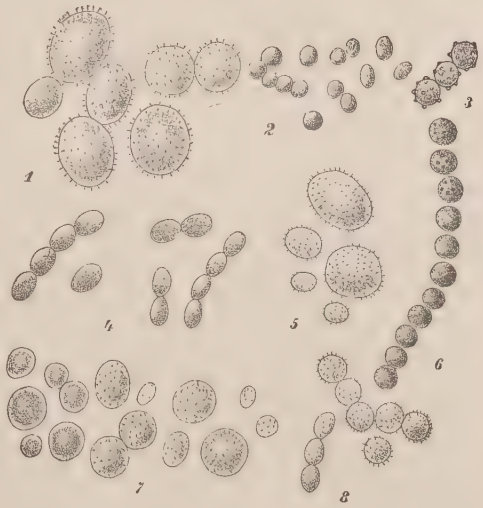


Fig. 164.—Conidia of Aspergillus.

All of approximately equal magnification, showing the differences in form and dimensions. *A. glaucus* (1), *A. fumigatus* (2), *A. niger* (3), *A. clavatus* (4), *A. Tokelau* (5), *A. varians* (6), *A. Oryzæ* (7), *A. Wentii* (8). Magn. about 1000. (Original.)

Up to the present, ascospores in the form of small globular nodules (Fig. 165) measuring about 60–300 μ in diameter, have only been definitely found in about five species (*A. glaucus*, *A. fumigatus*, *A. Rehmii*, *A. nidulans*, *A. pseudoclavatus*), but more will probably be discovered in time, in which event the form of these spores may be utilised as a basis of classification. Meanwhile it seems preferable to postpone the division of the genus *Eurotium* (*A. glaucus*) and to retain the conidiophores as a generic characteristic. In most cases the perithecia are fragile

capsules, with thin walls, and yellow, dark red, or even black in colour (*A. glaucus*, *A. pseudoclavatus*, *A. Rehmii*, *A. fumigatus*), which, in the last two instances, is enclosed in a shell formed of specially modified, coloured, thick-walled, swollen

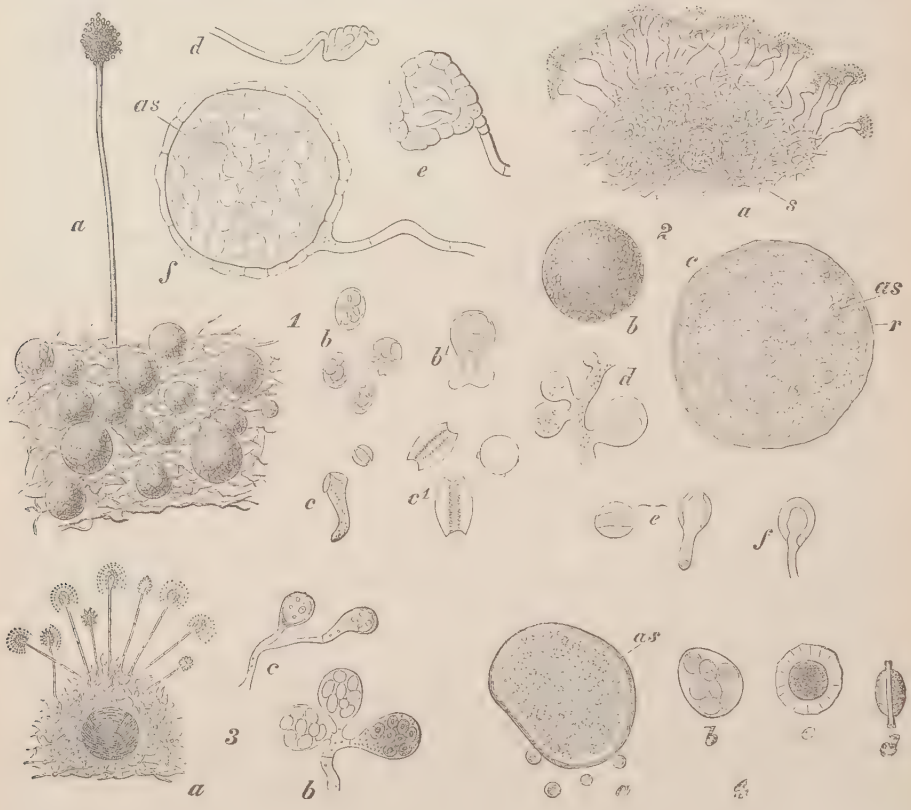


Fig. 165.—Ascospores of four species of Aspergillus.

Division 1.—Perithecia of *A. glaucus* resting freely on the substratum (*a*), also development of the perithecium from the *Eurotium* coil (*d*, *e*); at *f* a ripe perithecium with young asci; *b*, isolated asci; *c'*, ripe spores; *c*, the same germinating. (*c*, *b'*, *d*, *e*, *f*, *after de Bary*; *a*, *b*, *c'*, *after Wehmer*.) Magn. same as Fig. 169.

Division 2.—Sclerotia, with shell, of *A. nidulans* (*a*) prepared in the detached state (*b*); and section, showing sheath and asci (*c*); *d*, young asci; *e*, spores, one with germinating tube; *f*, swollen hypha of shell, with greatly thickened wall. Magn. same as Fig. 176. (*After Eidam.*)

Division 3.—Perithecia with shell, *A. Rehmii*, with separately prepared asci (*b*), and shell hyphæ (*c*). Magn. of *a*, about 100; of *b* and *c*, 1000. (*After Zukal*).

Division 4.—A Perithecium detached from the mycelial envelope of *A. fumigatus*, in section (*a*) containing asci (*as*); *b*, isolated ascus; *c* and *d*, spores with epidermal ridge, viewed from above (*c*) and from the side (*d*). Magn. same as Fig. 171. (*After Grijns*).

hyphæ, but in the others is naked. A shell is also found in *A. nidulans*, but here the ascospore is tougher, the asci (sclerotia), surrounded by a dark, stratified skin, being developed later. Similar naked or sheathed sclerotia, which, however, are sterile, were observed by Wilhelm (I.) in the case of *A. niger*, *A. ochraceus* and *A. flavus*. The development, chiefly through the implication and fusion of morphologically equal hypha, as in *A. Rehmii* and *A. ochraceus*

and also the character of the asci and spores, will be found compared in the description of the various species later. In *A. glaucus* the development proceeds from a single filament, in *A. nidulans* from two. The ascospores are therefore very unequal, a proper systematic appreciation of which difference would entail the establishment of different genera, the presence or absence of a separate shell being undoubtedly a generic characteristic. However, as already stated, this must be left out of consideration at present, unless weighty practical considerations be sacrificed to purely scientific points.

The following summary relates to species of *Aspergillus* which form perithecia or sclerotia :

A. Perithecia (with immediate formation of asci).

1. *A. glaucus*
2. *A. pseudoclavatus* } Perithecium naked without shell.
3. *A. fumigatus*
4. *A. Rehmii* } Perithecium with shell.

B. Sclerotia (asci formed after a while, or still unknown).

1. *A. nidulans*, with retarded formation of asci, and shell.
2. *A. ochraceus*
3. *A. niger* } Asci not yet observed. With or without simple mycelial sheath.
4. *A. flavus*

Eidam applied the name perithecium also to the tough-skinned organs of *A. nidulans*, which develops asci gradually, but also emphasises their intermediate position between the *Eurotium* capsules and the sclerotia.

Undoubtedly a large number of species will have to be struck out of the present list of about 120, the diagnosis made by older workers having been in many cases insufficient for the establishment of a new species. Their general practice was merely to describe and not compare, the latter having been difficult, owing to the scattered literature, previous to the appearance of Saccardo's *Sylloge*. Probably not more than two or three dozen are really admissible, a circumstance that unfortunately has not been duly considered by modern German workers, Lindau (I.), for instance, mentioning no less than 55 species, of which only 17 are classed as doubtful ; and only a small fraction of these have any interest for the technical mycologist. Nevertheless this genus is more important than the majority, since it comprises not only several species that find industrial application (*A. oryzæ*, *A. Wentii*, *A. luchuensis*), but also others noteworthy on account of chemico-physiological considerations (*A. niger*) and several which are pathogenic toward men and animals (*A. fumigatus*, *A. flavus*, *A. nidulans*), whilst others again (*A. glaucus*, *A. phœnicis*, *A. clavatus*, *A. fumigatus*) are occasionally found in industrial processes, commercial products, food-stuffs, &c. Whether certain species are directly pathogenic toward plants may be left out of consideration, though, according to Pammel, Weems, and Lawson-Scribner (I. *A. glaucus* and others are the cause of disease in embryo grasses. On the other hand J. Behrens (XVI.) found *A. glaucus* (=*A. medius*, Meissner) harmless, but *A. niger* dangerous. At all events, the genus *Aspergillus* forms the most interesting, because the most diversified, genus of fungi, except for the *Saccharomycetes*.

In the differentiation of species, the first point to consider is the colour of the herbage (young growth exclusively !) ; then the size and build of the conidiophores and conidia, and, finally, the physiological characteristics, such as food requirements, optimum temperature, energy of growth, special influences, &c. Moreover, the influence (if any) of the substratum—sugar, albumen, and also

gelatin—must be observed in each case. Differences that have not yet been fully appreciated also exist in the behaviour toward gelatin, and in the production of colouring-matters in the mycelium or nutrient solution, &c Attempts to identify old vegetative growths from the colour of the conidia, which is liable to a speedy change, especially in green species, are not to be recommended, the preparation of a young culture being essential, and other characteristics are liable to alteration as the cultures become aged. Many of the old *soi-disant* species undoubtedly owe their alleged existence to insufficient appreciation of these circumstances.

The conidiophore can in many cases be identified by the unaided eye, since it measures about 1–2 mm. in height (*A. niger*, *A. glaucus*, *A. oryzæ*, *A. clavatus*, *A. candidus*, &c.). Sometimes, under favourable conditions of growth, the length is nearly doubled (*A. Wentii*, *A. ochraceus*, &c.), whilst in adverse circumstances it may be considerably less (0.5–0.25 mm.). Such dwarf conidiophores are of common occurrence in otherwise luxuriant species (*A. oryzæ*, *A. candidus*, *A. glaucus*), accompanied by morphological modifications. Only a single species, *A. giganteus*, far exceeds the average height in its mucor-like conidial vegetation, the slender conidiophores averaging 1–2 cm. in length. Numerous species are characterised by the constant formation of small and very small conidiophores (*A. fumigatus*, *A. nidulans*, *A. minimus*, *A. Rehmii*, *A. spurius*, *A. flavus*), which cannot be detected as such by the unaided eye, except under favourable conditions, their length averaging less than 1 mm., and occasionally falling below 0.5 mm. (*A. fumigatus*, *A. minimus*, *A. Rehmii*), or even as low as 0.1 mm. (*A. fumigatus*), so that the nearly smooth surface growth closely resembles *Penicillium*.

The dimensions of the conidia vary between the limits of about 3 and 10 μ, the latter size being rarely exceeded. Some species invariably produce microspores exclusively, the conidia measuring only about 3 μ in diameter (*A. nidulans*, *A. minimus*, *A. fumigatus*, and frequently *A. niger*). The other extreme is reached by the species forming macrospores, with conidia measuring at least 5–6 μ, and often irregular in size (*A. glaucus*, *A. flavus*, *A. oryzæ*, *A. Tokelau*), attaining a diameter of 7–10, and sometimes as much as 15 μ, in the case of *A. glaucus* and *A. Tokelau*. An intermediate position in this respect is occupied by the species (*A. candidus*, *A. clavatus*, *A. Wentii*, *A. giganteus*, &c.) with conidia measuring about 3.5–5 μ, these being preferably classed with the *Microsporeæ*, the limit being fixed at 5 μ. In contrast to the conidia formed by successive constrictions of the tips of the sterigmata, and sometimes connected by delicate "intermediate cells," the primary sterigmata are formed by protrusions from the surface of the globule, sometimes before the stem has attained its full extension. The aperture of communication in the wall of the globule is but rarely (*A. giganteus*) visible under the microscope as a fine capillary channel (Fig. 163, *c*). The secondary sterigmata, first observed by Berkeley (in 1857) and Cramer (in 1860), are formed in succession from their parent cell.

Malformations are by no means rare in many species (*A. glaucus*, *A. oryzæ*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. fumigatus*, &c.), and have often been described. They include outgrowth of sterigmata into elongated tubes, vegetative hyphæ, and even into dwarf conidiophores; globular swellings of the vegetative hyphæ; irregular branching of the conidiophore apex, the globules being no longer formed; forking of the stem; abnormal branching of otherwise simple sterigmata, &c. It is sufficient here to merely record such (really unimportant) facts in order that they may be appreciated at their true value when observed. The occurrence of septa in the stem (especially in *A. flavus*), and sterigmata should probably be placed in the same category; at least this, and the often observed branching of the conidiophores, seem to be merely sports and not

constant characteristics, the conidiophore, as a rule, consisting of an unbranched, unicellular hypha.

It is also worthy of notice that many species flourish best at a high temperature (about 35°–40° C.), *e.g.*, *A. flavus*, *A. niger*, *A. oryzæ*, *A. clavatus*, *A. fumigatus*, *A. nidulans*, *A. Wentii*, a fact which may be utilised in the rapid differentiation of species. Among the kinds known at present, only *A. glaucus* and *A. candidus* exhibit a preference for low temperatures, though even many of the heat-loving species will pull through at very low temperatures (*A. niger* and *A. oryzæ* will grow below 10° C.).

A summary of the species may now be given, this being confined to the better known or more fully described (newer) kinds, omitting the numerous older and often unrecognisable ones. Those of technical importance are indicated by thicker type. It should be noted that the colour of the vegetation is not invariable, being influenced by the substratum, some green or white species, for example, occasionally becoming yellow, whilst, according to Vuillemin, the colour of *A. versicolor* ranges from green to red. The species in group 4 undoubtedly include several synonyms, and also the white (2) and blackish brown (3) kinds require elucidation, so that in reality only the green species can be regarded as anything like properly established.

Summary of the Aspergillus Species grouped according to the Colour of the Conidial Vegetation, the Character of the Sterigmata, and the Existence of Ascospores.

1. Green (grey, bluish green or yellow-green), viz. :
   (*a*) With simple sterigmata. **A. glaucus**, Link, with ascospores (naked perithecia) ; *A. clavatus*, Desmazières ; **A. fumigatus**, Fresenius, ascospores (cased perithecia) ; **A. oryzæ**, (Ahlburg) Cohn ; *A. varians*, Wehmer ; *A. minimus*, Wehmer ; **A. flavus**, Link, with sterile sclerotia ; *A. giganteus*, Wehmer ; *A. cæsiellus*, Saito ; *A. Tokelau*, Wehmer ; *A. penicillopsis* (Hennings), Raciborski.
   (*b*) With branched sterigmata : *A. nidulans*, Eidam, ascospores (ensheathed sclerotia) ; *A. pseudoclavatus*, Puriewitsch, ascospores (naked perithecia) ; *A. variabilis*, Gasperini ; *A. versicolor*, Vuillemin.
2. White, viz. :
   (*a*) With branched sterigmata (associated with simple sterigmata in the case of *A. candidus I.* ; *A. candidus I.*, Wehmer ; *A. albus*, Wilhelm.
   (*b*) With simple sterigmata: *A. candidus* (Link), Saccardo.
3. Blackish brown, viz. :
   (*a*) With branched sterigmata. **A. niger** (Cramer), van Tieghem, with sterile sclerotia ; *A. phœnicis*, Fat. and Delacr. ; *A. strychni*, Lindau ; *A. pulverulenta*, MacAlpine ; *A. atropurpureus*, Zimmermann ; *A. violaceo-fuscus*, Gasperini.
   (*b*) With simple sterigmata. **A. luchuensis**, Inui ; *A. calyptratus*, Oudemans.
4. Brownish-yellow, yellow, brown and reddish, viz. :
   (*a*) With simple sterigmata : *A. ostianus*, Wehmer ; **A. Wentii**, Wehmer ; *A. perniciosus*, Inui ; *A. giganteo-sulfureus*, Saito ; *A. citrisporus*, von Höhnel.
   (*b*) With branched sterigmata (occasionally associated with simple ones). *A. sulfureus*, Fresenius ; *A. ochraceus*, Wilhelm (with sterile sclerotia) ; *A. Rehmii*, Zukal (with ensheathed perithecia) ; *A. spurius*, Schröter ; *A. elegans*, Gasperini ; *A. auricomus*, Guéguen (with sterile sclerotia).

§ 284. Aspergillus Species with Simple Sterigmata.

Aspergillus oryzæ, (Ahlburg) Cohn (= *Eurotium oryzæ*, Ahlburg). This species is of practical importance as a saccharifying fungus, and has been cultivated for centuries in Japan for the preparation of the rice mash for Saké, as well as for the production of Soja sauce and Miso. It was first identified (as *Eurotium oryzæ*) by Ahlburg (I.) in 1876, and was renamed *Aspergillus oryzæ* by Cohn (XIII.) in 1883, after which it was examined by Büsgen (IV.) though the full morphological description—by Wehmer (VIII.) —was not given until

1895. The species illustrated in Fig. 166 produces a luxuriant mould vegetation, which is usually yellow-green (rarely yellow), with large, closely set tough conidiophores about 2 mm. high. It grows rapidly on a large variety of liquid and solid media, and is easily cultivated even at room temperature, the optimum temperature being above 30° C. After several weeks, or even months, the colour sometimes gradually turns brown. The peculiarities of the conidiophores, sterigmata and conidia enable the species to be distinguished with comparative ease from most others, *A. flavus* alone being similar. The clavate or spherical globule, which varies in size and shape, usually exhibits no definite line of demarcation from the smooth or finely granular, pale stem. The sterigmata are radial —or in small conidiophores—confined to the summit and pointing upward; slender, simple, large, yellowish green, spherical conidia (6–7 μ thick, smooth or finely granular), undergoing constriction into chains which rapidly fall asunder; the size and form, however, vary considerably. The green heads, which may measure over 100 μ across, with globule up to 80 μ in diameter, though often much smaller, appear in all sizes. The sterigmata on well-developed heads measure 12–20 by 4–5 μ, and therefore differ greatly from the short, compact sterigmata of *A. glaucus*. No ascospores or sclerotia have yet been observed; and the same applies to budding cells (the alleged Saké "yeast"), which, though frequently stated to exist, have never yet been described with any precision. Malformations of the conidiophores (forked stem, outgrowth of sterigmata to filaments or delicate conidiophores, and also branching) are not infrequent. This species secretes a very active diastase (*see* § 290); it has been recommended and tried as a malt substitute in Europe as well as in the Orient, Korschelt's first report (II.) in this connection (1876) having been succeeded by a number of chemico-physiological investigations and technical communications on this plant which has been cultivated in Japan from time immemorial—compare Wehmer's compilation (XVII.). According to the latter authority, the conidia remain capable of germinating for years. Hiller (I.) states that the substratum on which the fungus is grown has an influence in this connection, certain nutrient media (wort) being favourable, whilst others (dextrose) are the reverse. Fuller morphological particulars are given by Cohn (XIII.), Büsgen (IV.) and Wehmer (XVII. and VIII.).

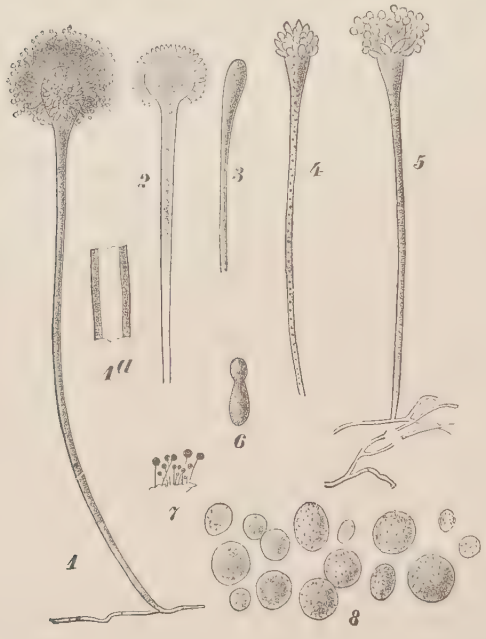


Fig. 166.—Aspergillus oryzæ.

1–2. Conidiophores with clavate and almost spherical globule. 2. In optical section. 3–5. Development of a small conidiophore, distension of the hypha, protrusion of sterigmata and incipient formation of conidia. 1a. Optical section of tough stem. 6. Sterigma. 7. Conidial herbage, slightly magnified. 8. Conidia. Approximate magn. of 1–5, 75; of 6, 400; of 8, 900. (*After Wehmer.*)

Aspergillus Wentii, Wehmer, was observed by Went in the preparation of Tao Yu (*see* vol. i. p. 248) according to the method practised in Java, and was described

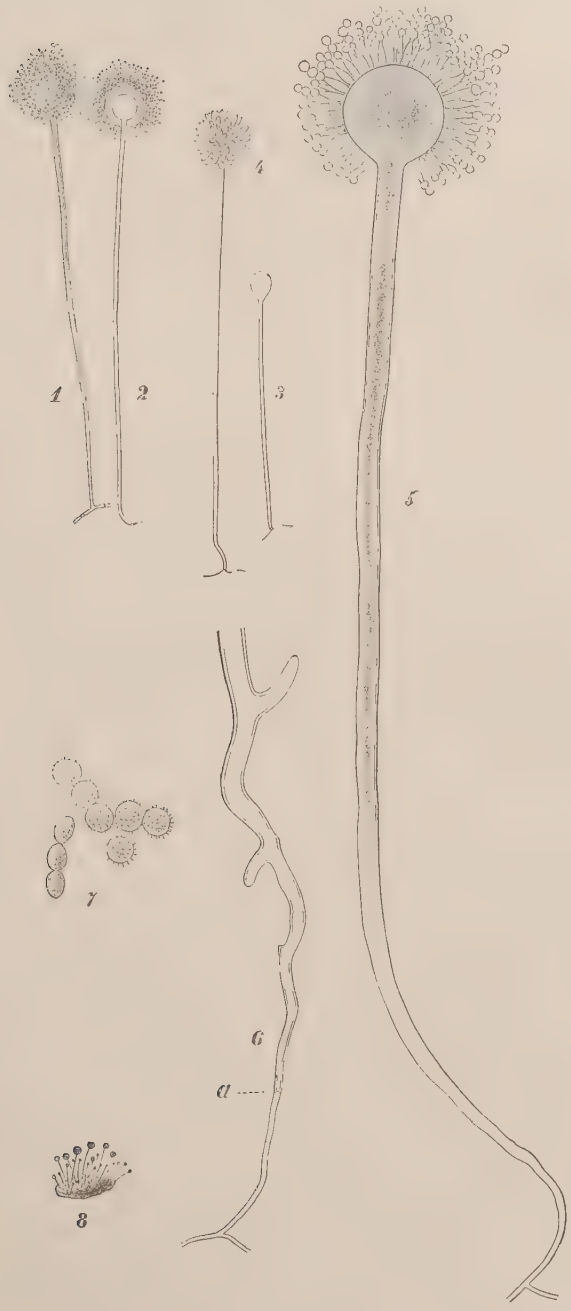


FIG. 167.—Conidiophores (1–5), 2 and 5 in optical section. Figs. 3–5, development of the globule (3) and sterigmata (4). The separation of the conidia is beginning in 5. 6. Base of conidiophore, with lateral protrusions. Conidia (7), young herbage (8). Approximate magn. of 1–4, 20; of 5 and 6, 120; of 7, 900. (*After Wehmer.*)

by Wehmer (XIX.) in 1896. It appears spontaneously on the boiled Soja beans that have been covered with *Hibiscus* leaves, and effects a loosening and disintegration of the firm tissue of the bean. The species forms a pale coffee-coloured, dense mould vegetation (Fig. 167), with conspicuous conidiophores, about 2–3 mm. in height, their thick brown heads (up to 200 μ in diameter) showing up clearly on the pale, slender, tough-skinned, smooth stalks, and being quite unmistakable for any other species. The decidedly spherical globule (75–90 μ in diameter), sharply contrasting with the stalk, is covered on all sides with a dense growth of slender radial, simple sterigmata (mostly 15 by 4 μ), from which the small, coloured, globular to elongated, finely punctated or smooth conidia (about 4–5 μ in diameter) separate by constriction. The mycelium, which is snow-white, though sometimes red—and in old cultures reddish brown—when grown in closed culture vessels attains a considerable height above the substratum, and also throws up a large number of conidiophores under these conditions (*see* Fig. 168). No perithecia or sclerotia have yet been discovered. This quick-growing species, which can be easily cultivated on the usual mycological substrata, flourishes particularly well in the incubator (above 30° C.). Nothing is known about the enzyme, which plays an active part in the decomposition of the Soja bean. Like the preceding species, this fungus does not belong to the European flora, though both will grow well here.

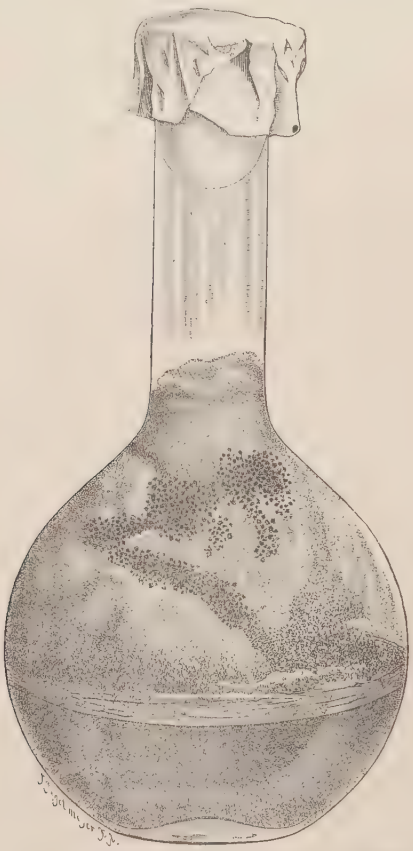


Fig. 168.—Aerial mycelium of *Aspergillus Wentii*, with conidiophores, growing rank in a culture flask. About natural size. (*After Wehmer*).

Aspergillus glaucus, Link (*Eurotium Aspergillus glaucus*, A. de Bary), is the ordinary green mould, which grows everywhere, especially on dried plants, old black bread (*Pumpernickel*), skins, jam, old leather articles, herring pickle and other materials. This species has long been known, and is met with in the literature under various names: *Eurotium herbariorum*, Link; *Aspergillus herbariorum*, *Eurotium Aspergillus glaucus*, de Bary; *Eurotium glaucum* (*E. repens* also seems to be the same fungus). A. de Bary (IX.) in 1859 identified the ascospores (VIII.) of the so-called *Eurotium herbariorum* with the conidiophore form of *Aspergillus glaucus*, Link, and showed the two to be one and the same fungus. The young conidial herbage is pale green to verdigris-coloured, but darkens quickly to a dirty greyish green or greyish brown, the mycelium also changing colour by the deposition of pigment granules, and becoming pale yellow, which turns a dirty rust-brown. Consequently, old vegetations are often entirely discoloured and ugly, new sowings being requisite for the identification of the fungus. Hence culture experiments are necessary, the characteristics of old growths being unreliable. Many of the

different earlier *Aspergillus* species, established on the basis of such material are probably nothing more than old vegetations of *Aspergillus glaucus*, and should be struck off the list. Some herbages exhibit conidia exclusively, whilst others produce only numerous golden yellow perithecia (for example, on cranberry jam). The conidiophore shown in Fig. 169 (1–3 mm. high) is readily distinguishable from other species; the globule, sterigmata and conidia present characteristic features. The globule, which is not sharply demarcated from the stalk, is spherical to knob-like, measuring about 60 μ across, and thickly covered all over with very short, simple sterigmata (up to 14 μ by 7 μ), dividing into

FIG. 169.—Aspergillus glaucus.

Conidiophores (1, 2), sterigmata (3) and conidia (4). A portion of mycelium, with overlying perithecia and a conidiophore (magnified) is shown at 5, whilst 6 gives the natural size. Sections of perithecia are given at 7, with young asci (*as*) and first stages of development (*Eurotium coil*); isolated asci (8) and detached spores (9), germinating at *c*. Approximate magn. of 1–2, 50; of 7, 170; of 8, 260; of 9c, 700. (1, 7, 8, 9 (in part) *after de Bary*, the rest *after Wehmer*.)

unusually large, prickly, globular or slightly elongated conidia (7–30 μ and more in diameter). These latter are remarkably large in proportion to the sterigmata (about half as broad as these are long), which are plump, their length being only about double the width, and not slender and pointed as in many other species. *A. glaucus* has larger conidia than any other well-known species, and none of the others produces perithecia with such readiness and abundance. These are small (about 100–200 μ in diameter), pale brown-yellow-coloured at first, afterwards ugly brown capsules with a simple, delicate envelope, and enclosing numerous rounded oval asci. Each ascus contains 5–8 colourless, smooth, ellipsoidal spores, exhibiting a longitudinal furrow and measuring 7–10 μ long by 5–8 μ broad. The spore wall bursts open and ejects the exospore, which germinates to a new mycelium. The gradual development of the perithecia from spirally coiled hyphæ need only be briefly mentioned here, having been already described in

the majority of botanical works. This proceeds both in the presence and absence of light, and therefore—as in the formation of conidia in *A. niger*, &c.—is not hindered by the action of light, as was assumed by Elfving (I.). The same author's assertion respecting the formation of yeast cells is also dubitable and lacking proof. According to Lindner (II.) the conidiophores can be made to branch abundantly by restricting the food-supply or adding antiseptics.

In point of substratum this industrial and domestic fungus is selective, since it does not thrive on (*e.g.*) liquid saccharine media with mineral salts and inorganic nitrogenous food, whereas black bread or—as a good bacteriological substratum—wort gelatin, is favourable. The fungus also prefers moderate temperatures (it will grow at 8°–10° C.) and ceases to develop at blood temperature, consequently its alleged occasional appearance in the human ear—recently mentioned again by Hatch and Row (I.)—is probably a mistake arising from the species having been confused with *A. fumigatus* or *A. flavus*. Nomura (I.) states that it is associated with *A. flavus* in the cocoon fungus ("Uchibaki") which does so much damage to the silk industry, and was first attributed to *Aspergillus* species by Raux (I.). It is certainly a chief source of mould in black bread, and is stated by J. Behrens (III.) to be a frequent, injurious dweller in "shed-ripe" tobacco and cigars (*see* vol. i. p. 128), as well as in hops. According to Spieckermann and Bremer (I.) it is the cause of mould in cotton-seed meal; and perhaps it is among the still undescribed species of *Aspergillus* that damage leather. Aderhold (IV.) found it in acid gherkin pickle; and it thrives on smoked meats (ham), preferring very dry substrata. Whether, in certain cases, it is actually pathological toward plants and takes part in the blackening and spoiling of chestnuts, as was stated by Roze (I.), still remains to be fully investigated Occasionally it is found on the kernels of walnuts and hazel-nuts still in the shell, an abundance of perithecia being produced. The limits of temperature for this species are 7°–37° C., the optimum being 27°–29° C. according to Klebs (I.), though others give the optimum at 20°–25° and the maximum as 30° C. (*Elfving, Siebenmann*). More complete morphological data are furnished in the works of A. de Bary (IX.), Wilhelm (I.), Siebenmann (I.), R. Meissner (I.), and Wehmer (XVII.).

The fungi termed *Eurotium repens*, de Bary, and *E. Aspergillus medius*, Meissner (I.), are presumably the same as *A. glaucus*, since there are no tangible differences between them exceeding the usual limits of variation. It is, nevertheless, highly desirable that this doubtful point should be finally settled by careful investigation. Whether the *Eurotium rubrum*, Spieckermann and Bremer (I.), found in mouldy cotton-seed meal is a different species also seems questionable, and requires elucidation.

Aspergillus flavus, Link, greatly resembles *A. oryzæ* in the yellowish-green superficial colour of the herbage, and also in the shape of the conidiophores; but is readily distinguishable by the smaller dimensions of the latter (less than 1 mm. high). This species, which has been identified as pathogenic in animals, has a preference for warmth, the optimum temperature being about 37° C. It is frequently observed in cases of mycosis of the human ear (where it is sometimes confounded with *A. glaucus*), and also occurs on bread, portions of plants, and dried excrement. Even at blood temperature it thrives luxuriantly on all kinds of mycological substrata, and rapidly produces extensive yellow-green growths of mould. The superficial colour is rarely pure yellow, and the older growths (several weeks or months) are very liable to turn colour, becoming finally an ugly dark brown. The conidiophores (Fig. 170), which generally measure less than 1 mm. (0.5–0.7 mm.), carry a spherical or club-shaped globule, which rarely springs in a sharply defined manner from the pale, warty stem; and the simple, slender sterigmata, which are generally disposed radially,

though sometimes confined to the summit, develop large conidia (average 5–6 μ in diameter), which are generally of an irregularly globular shape, and smooth (sometimes finely granular), separate, by constriction, into chaplets, which readily become dissociated. The coloured heads measure up to about 90 μ, the globules 30–40 μ in diameter, the sterigmata usually about 20–60 μ. The conidia vary between 4 and 8 μ in diameter, but in any event the species (with *A. glaucus* and *A. oryzæ*) belongs to the large-spored class. No perithecia have been

FIG. 170.—Aspergillus flavus.

Conidiophores with spherical to club-shaped globules and simple sterigmata (1–4), the outer wall of the (frequently septate) stem being roughened by colourless granules (5). 6. Conidial herbage (about 2/1). 7. Conidia. Magn. of 1–4, 140; of 5, 400; of 7, 500. (*After Wehmer.*)

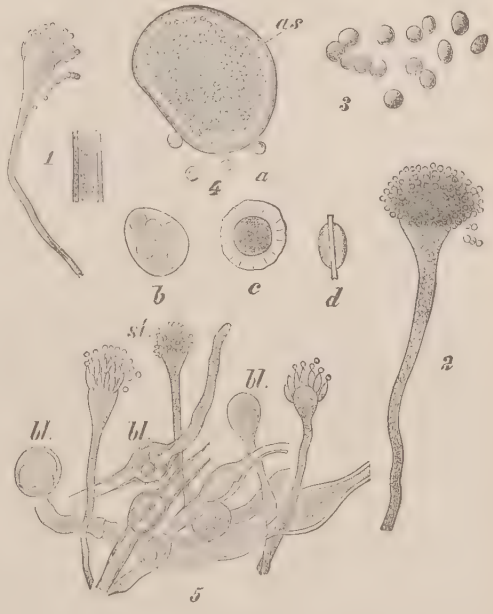


FIG. 171.—Aspergillus fumigatus.

1-2. Club-shaped conidiophores (in optical section at 1). 3. Conidia. 4. Asccus and ascospores. 5. Hyphæ (*bl*) with peculiar globular swellings, and conidiophores, from herbage. Approx. magn. of 1, 2, 5, 140; of 3, 1000; of 4*a*, 70; of 4*b*, 719; of 4*c* and 4*d*, 2250. (4*a–d after Grijns*, the rest *after Wehmer.*)

observed, but WILHELM (I.) in 1877 described small, black, nodular sclerotia (about 0.7 mm. in diameter), with a thick skin and pale core, which remained sterile in germination tests. These appear to be formed by simple intertwining and fusion of morphologically uniform filaments. Further morphological details are furnished by WILHELM (I.), SIEBENMANN (I.), and WEHMER (XVII.).

According to NOMURA (I.), this fungus is the chief source of injury in the cocoon disease of silkworms, but does not play any other industrial *rôle*, though sometimes found as a subordinate fungus on mouldy cotton-seed meal. In the literature it is often, but erroneously, classed as *A. flavescens*, Wred., a species which should be struck out as merely synonymous; and, in view of the lack of perithecia, de Bary's name, *Eurotium A. flavus*, is also inappropriate. The *A. subfuscus*, JOHAN-OLSEN (III.), still encountered in the literature, is probably only *A. flavus*.

Aspergillus fumigatus, Fresenius, a cosmopolitan green to greyish green (not yellow-green) species, characterised by a high optimum temperature (about 40° C.) and rapid growth, is chiefly of medical interest (pathogenic), but occasionally acts injuriously in industrial processes carried on at high temperatures, such as certain fermentations (*e.g.*, lactic fermentation). According to Behrens, it occurs on the ribs of fermenting tobacco leaves (*see* vol. i. p. 129); and on one occasion Wehmer (I.) also found it, in the form of large patches, on woollen fabrics. It likewise attacks vegetables (decaying potatoes, bread, malt, beer wort, &c.) in the incubator; and, according to F. Cohn (XIV.), it also produces a thermogenic effect (*see* vol. i. p. 126). Of greater importance is its frequent occurrence in the body cavities of men and animals (*e.g.*, the human ear and the lungs of various birds), where it produces otomycosis and pneumomycosis, which latter malady, according to Rénon (I.), is almost invariably found among workers in certain trades, such as pigeon-fatteners and hair-combers (in Paris). Conidia introduced into the arterial circulation of animals germinate in the body and produce serious illness, which has mostly a fatal termination. The species was first discovered by Fresenius (I.), in 1841, in the bronchi and air cavities of a bustard. The conidial herbage, however, is not—as the name would imply—smoky grey, but penicillium-green, though quickly turning to grey and even to dirty brown. It is readily identified by the dwarf conidiophores (Fig. 171) 0.1–0.3 mm. long, with club-shaped globule (10–20 μ), thick, simple, slender, upright sterigmata (6 15 μ long), grouped on the crown, and with long chains of very small (2–3 μ) conidia, mostly globular. Hence, racial variations apart, the species cannot easily be mistaken for any other. Though J. Behrens (XIV.) mentioned the occurrence of perithecia, and Siebenmann (I.) sclerotia, we are indebted to Grijns (I.) for a description of the true perithecia. According to this worker, they are small, globular, nut-brown bodies, measuring 250–350 μ in diameter, with a special integument, from which the true, dark red perithecium (which has a very fragile, stratified coloured wall) can be extracted without difficulty. The interior consists of a colourless network of filaments, surrounding a number of colourless, oval, thin-skinned asci (14–9 μ), each of which contains eight red lenticular, tough-skinned spores (4–4.5 μ), surrounded by a pale, radially striped equatorial ledge. Hence a considerable difference exists between the frontal and lateral appearance of these spores, which, moreover, do not become coloured until shortly before maturity. These asci, which recall those of *A. nidulans* (Eidam) in the appearance of the shell, were found in large numbers by Grijns on the surface of the herbage and a number of cultures derived therefrom. Owing to the resemblance of the ascospores to those of *A. nidulans*, Vuillemin (I.) considered—which is hardly probable—that Grijns was really dealing with the last-named species. Further morphological details are furnished by Fresenius (I.), Siebenmann (I), Behrens (III.), Weimer (XVII.), and Grijns (I.). *A. nigrescens*, Rob., and *A. bronchialis*, Blumentritt (I.), appear to be synonymous with *A. fumigatus*, though Blumentritt (II.) recently confirmed the existence of small variations from the cultures of *A. bronchialis*. Costantin and Lucet (II.) wish to subdivide *A. fumigatus* into a number of forms differing in part by their pathological behaviour; and they also describe new allied species: *A. Lignieresi* and *A. virido-griseus*. With regard to races of *A. fumigatus* and pathogenic species, compare Savoff (I.), Savouré (I.), Bodin (I.), Guéguen (III.), and Macé (II.).

Aspergillus luchuensis, Inui. According to Inui (III.), this recently described mould fungus plays a similar part in the preparation of "Awamori"—a beverage resembling whisky—in the Loochoo islands to that filled by the rice *Aspergillus* in making Saké, is similar, morphologically, to *A. Wentii*, though more like *A. niger* in colour. The conidiophores (Fig. 172), which are 1–2 mm. in height,

develop blackish brown heads (40–80 μ thick), whose spherical (rarely knob-like) globules (20–30 μ in diameter) are thickly covered with radially arranged, conical sterigmata, bearing spherical conidia (4–5 μ thick), covered with tiny wart-like protuberances. No perithecia have been observed up to the present. This species saccharifies starch—in which respect it deserves further investigation—and is very similar to *A. Wentii*, apart from its colour and somewhat smaller dimensions. The optimum temperature of growth is between 30° and 35° C.

Associated with this species in Awamori koji, Inui (III.) found another, which is morphologically analogous, but of a greyish brown colour (*A. perniciosus*), which,

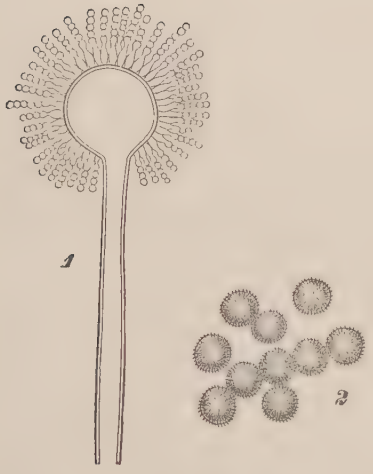


Fig. 172.—Aspergillus luchuensis.
Conidiophores and conidia. Approx. magn. of 1, 300; of 2, 1000. (*After Inui.*)

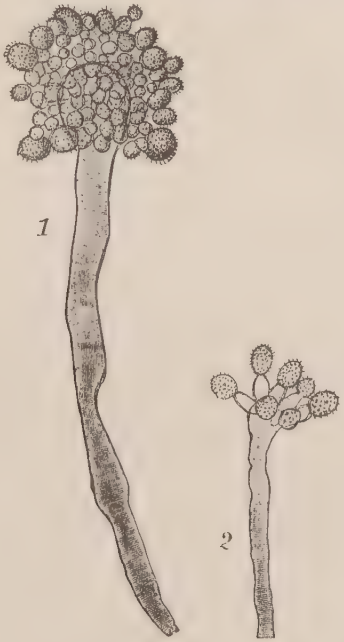


Fig. 173.—Aspergillus Tokelau.
Conidiophores of different sizes from diseased human skin. Approx. magn. 300. (*After Wehmer.*)

however, is merely regarded as an impurity, and is subordinate, or entirely absent, in good koji.

Aspergillus Tokelau, Wehmer. This species was discovered by Tribondeau (I.) in the infectious "Tokelau" or Samoa disease, attacking the natives of certain of the Pacific islands. It was named "Lepidophyton" at first, and is chiefly of medical interest, though worthy of note as parasitic on the human skin. Whether the species plays a more comprehensive part in that disease (formerly known as "Trichophytis") remains to be ascertained; but at any rate the fungus is a true *Aspergillus*, and indeed, according to Wehmer (XIX.), a well-defined new species, characterised by large hairy conidia (up to 12 μ in diameter), resembling those of *A. glaucus* (*see* Fig. 173), growing on conidiophores which at times are like those of *A. glaucus*, and at others those of *A. fumigatus*.

Aspergillus clavatus, Desmazières, a species often found on vegetables, and, according to P. Lindner (XXXIII.), more especially on green malt, on which it forms a pure green (not yellow-green!) herbage, is otherwise devoid of practical

importance. It is distinguished by the peculiar elongated globule (similar to a gun sponging-rod, and measuring 150 μ by 35 μ), such as are observed in *A. pseudoclavatus* (with branched sterigmata) and *A. giganteus;* and was described by Desmazières (I.), in 1834. The short, simple sterigmata (about 8 μ by 3 μ)—*see* Fig. 174—divide by constriction into small, oval (not globular!), smooth conidia (4.2 by 2.8 μ) in long chains, enveloping the elongated heads with a greyish green dust. The length of the conidiophore stems, which are 15–25 μ thick, reaches about 2 cm., but seldom exceeds that figure.

Fig. 174.—Aspergillus clavatus.

Conidiophores in various stages of development, with elongated globule and simple sterigmata, shown in optical section at *c*, with incipient conidia at *e*. A slightly magnified herbage is seen at *f*. Conidia (*g*), sterigma (*h*), section of stem (*i*). Approx. magn. of *a* and *b*, 30; of *c* and *d*, 60; of *e*, 120; of *g* and *h*, 1000. (*After Wehmer.*)

Other species mentioned in the literature as occurring on plants are the large, greenish yellow *A. penicillopsis* (Hennings), Raciborski (II.), as well as the dwarf, olive coloured *A. Delacroixii* (Delacroix), Saccardo and Sydow, observed by Delacroix on cocoa beans. Both these species deserve further investigation. Among other well-known species we will only mention the green *A. varians*, Wehmer, *A. minimis*, Wehmer (observed on leaves and on sugar solutions), and *A. ostianus*, Wehmer (found on leaves and boiled rice), which latter species is characterised by a pale ochreous pigment. There are also a number of more or less completely described species, chiefly observed on vegetables, and for the most part not yet cultivated, including the new *A. calyptratus* and *A. Koningi*, Oudemans (II.) and *A. citrisporus*, F. von Höhnel (I.).

As a giant among its kind, mention may also be made of *Aspergillus giganteus*, Wehmer (XVII.). The conidiophores of this fungus (which grows on sour wort)

resemble those of *A. clavatus* in the shape of the globules and sterigmata, and attain an average length of 1–2 cm., and therefore about ten times that of most of the other species. Before the heads begin to colour, the herbage has a mucor-like appearance, and it is only later that it is clearly distinguishable from the greyish yellow or dark mucor vegetation by the greyish green tinge of the conidial heads (1000 by 120–125 μ) on their slender, pale, saffron-yellow stems. The short sterigmata (9–12 by 4–5 μ), which are invariably simple and thickly cover the surface of the globule (500–800 μ by 80–100 μ), produce comparatively small, smooth, oval conidia, measuring on the average 4 μ by 2.6 μ. The species thrives on the usual substrata at room temperature and is easily cultivated. A noteworthy feature is the readily detectable perforation of the wall of the globule below the sterigmata, in the form of a narrow channel (when viewed in section, and a tiny circle when viewed in plan) appearing inside the larger one corresponding to the diameter of the sterigma, and causing the globule to seem as though covered with small concentric circles (*see* Fig. 163, 11). No perithecia have yet been observed.

§ 285. Aspergillus Species with Branched Sterigmata[1] (Sectio Sterigmatocystis).

Aspergillus niger, van Tieghem = *Sterigmatocystis antacustica*, Cramer = *Sterigmatocystis niger*. This well-known and widely spread species, which has been frequently studied from a chemico-physical standpoint and has a literature of its own, can be identified by the brownish black conidial herbage, with imposing, stiff, slender conidiophores, several millimetres in height. In any event, every *Aspergillus* of this colour described in the literature under some other name needs careful identification. Specific names, such as *A. nigricans*, Wreden (1869); *A. nigrescens*, Robin (1851); and *A. nigricans*, Cooke, should be abolished entirely; and at least half a dozen others are in the very doubtful class. The proper specific name *pro tem.* is *Sterigmatocystis antacustica*, which was given by Cramer (II.) to the fungus he discovered in the passage of the human ear in 1859. As was shown by Wilhelm (I.), the fungus afterwards (1867) termed *A. niger* by van Tieghem coincides with the above species by also possessing branched sterigmata. The morphological examination of the structure of the conidiophores (Fig. 175), which necessitates the removal or bleaching of the dark masses of conidia, reveals a pale, rigid stem, about 15 μ thick, carrying a sharply defined spherical globule (diameter about 80 μ), with slender radial primary sterigmata (26 μ by 4.5 μ) each with 3–4 ornamental secondaries (8 by 3 μ), and long chains of small globular, smooth or warty conidia (about 3–4 μ) as carriers of the dark colour. Moreover, the reports of various authorities do not altogether agree, the dimensions of the conidia being oftentimes given as 3.4–4.5 μ, and the length of the sterigmata as 20–100 μ. This must be specially emphasised, in view of the diagnosis of the black species, to be described later. Of course the heads vary in size, and the result obtained depends on which of them have been measured, unless the average be taken. In unfavourable circumstances, for instance, unsuitable media, the conidiophores languish (few sterigmata and simple, conidia pale, &c.), as was observed by Duclaux (XX.), and more recently by Molliard and Coupin (I.) as well as by Lutz (II.). C. Engelke (I.) states that, under certain conditions, a conidial form, similar to that of *Botrytis*, *Sceptromyces Opizii*, Corda, is produced; but this somewhat improbable report requires confirmation on the basis of indubitably pure cultures.

[1] The division is by no means sharp, some species exhibiting both simple and branched sterigmata.

Sclerotia have been frequently observed in this fungus, the first to discover them being K. Wilhelm (I.) in 1877; but unaccompanied by any development of asci. According to Brefeld (IV.), they are formed by the simple intertwining and fusion of morphologically equal hyphæ and take the appearance of yellowish, hard, tough-skinned and nearly globular nodules, 1–3 mm. in diameter. They lie scattered over the surface of the herbage—inside as well, according to Wilhelm (I.)—but are rare, and only found occasionally. This easily cultivated fungus, which, though a lover of warmth, will continue to grow slowly a few degrees above freezing-point (optimum temperature about 40° C., minimum about 7° C.), and exhibits a preference for certain acid substrata (gall-nut extract, solutions of tannic acid, and also solutions of fruit acids containing sugar and other nutrient substances)—in consequence of which it is said by Wehmer (XXIII.) to be easily captured—is of little practical importance. On the other hand, it is all the more important as an experimental fungus in the study of fungo-physiological questions. It plays a practical part in the preparation of gallic acid from tannin, and also in opium manufacture (compare § 292). The physician is acquainted with it as a not infrequent inhabitant of the human ear, in otomycosis, though its appearance in this case seems to be secondary. Its share in the retting of flax is doubtful. According to Bordas (II.), it is the cause of the cork disease (known in France as *piqûre* or *tache jaune*) affecting many cork oaks on the side exposed to the weather. The bottle corks made from this diseased material are liable to impart a corked taste to wine, and contain the *Aspergillus*, either alone or associated with other mould fungi. Behrens (XVI.) states that it not infrequently exerts an injurious action in germination tests on seeds, inoculation tests having resulted in stunting the embryos of numerous species, so that a pathological character is assumed in these circumstances. The numerous chemical influences of this fungus, including the very decided capacity for producing oxalic acid, are described more fully in chapter lvii. Linossier (II.) states that the, presumably ferruginous, black pigment (the so-called aspergillin) is of some physiological importance in the life of the plant; but this is doubtful in view of its nature as an excretion product of the conidia, although both Molisch (I.) and Kanter (I.) assert that iron is indispensable for the fungus. Greater interest seems to attach to the yellow pigment in the hyphæ, examined by Milburn (I.). Here we can only refer briefly to a whole series of recent investigations by Czapek (III.), R. Chodat and Bach (I.),

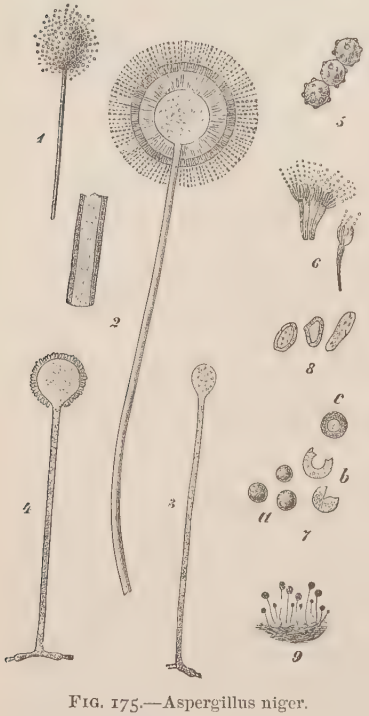


Fig. 175.—Aspergillus niger.

1 and 2, Conidiophores, in optical section at 2, after decoloration and clearing, showing the spherical globule and double radial wreath of branched sterigmata, as well as the conidial zone (semi-diagrammatical); 3 and 4, young conidiophores before and during the production of sterigmata (optical section); 5, globular, warty conidia; 6, separately prepared sterigmata; 7, sclerotia, after unsuccessful germination experiment (fallen apart at *b*); 8, tough-skinned spotted cells from the interior of the sclerotia; 9, conidial herbage. Approx. magn. of 1–4, 40; of 5, 1000; of 6, about 154; of 7, natural size; of 9, about 2. (*After Wehmer.*)

Raciborski (II.). Lode (I.), Ono (I.), Hattori (I.), Kny (II.), Bourquelot and Hérissey (II.), Saida (I.), Iwanoff (I.), Kosinski (I.), Richter (I.), Emmerling (V.), Lutz (II.), Friedel (I.), Maximow (I.), Kostytschew (I.), Koernicke (I.), Kanter (I.), Heinze (II.), Jousset (I.), Orlowski (I.), Molliard and Coupin (I.), Kurzwelly (I.), Kosjatschenskow (I.), Lesage (IV.), Pantanelli (I.), Altenburg (I.), Charpentier (I.), Krasnosselsky (I.), E. Meissner (I.), Porodko (I.), R. Meissner (IV.), Todur (I.), Garnier (I.), and Coupin (I.), dealing with the chemical composition, nutrition, respiration, the production of enzymes, influence of stimulants, radiation, resistance of the conidia to injurious influences, &c. The literature on this fungus previous to 1901 has been collected by Wehmer (XVII.), who gives no fewer than 79 references. The numerous enzymes produced by this fungus are dealt with in the next chapter.

Fungi allied to *A. niger* occasionally inhabit the interior of certain fruits. Thus, Corda found in dates a species which he named *Ustilago phœnicis*—the *Aspergillus phœnicis* of Patouillard and Delacroix (I.), who identified it as a species of *Sterigmatocystis*, and Hennings (II.) also recognised as a *Sterigmatocystis* (*St. ficuum*), the *Ustilago ficuum* discovered by Reichardt (I.) in dried figs. According to G. von Lagerheim (I.), the two have since been found to be identical. The question now arises whether this date and fig fungus, which has not yet been compared, in pure cultures, with *A. niger*, is really different from the latter. This point is by no means clear; and a short description of the fungus may be given here, on account of its injurious effect on the fruits in question. According to Hennings (II.), the conidiophores fill the interior of the figs with a compact black mass of conidia, and their heads measure 76–100 μ in diameter, the globule being 45–60 μ across, and closely set with club-shaped primary sterigmata (15–28 by 6–9 μ). The dark, slender sterigmata (5–8 by 2–3 μ), mostly present, produce long chains of globular, blackish violet conidia, usually 4 μ thick, which are said by Hennings to be smooth, but which G. von Lagerheim (I.) asserts to be provided with granular ledges. According to the latter authority, sclerotia are also formed. The fungus produces oxalic acid, saccharifies starch and inverts saccharose, all of which properties are found in *A. niger*, and the dark pigment of the conidia behaves in the same manner. The date disease ("Mchattel") caused by this organism is of frequent occurrence in the valley of the Nile. Hennings states that gastric troubles ensue when figs affected by this fungus are eaten. The species is indigenous to Egypt and Tunis (in dates and figs).

A similar species, *A. strychni*, has recently (1904) been described by Lindau (I.), as filling with black conidial powder the mummy-hard masses of the dried fruit of *Strychnos leiosepala* in Angola. The stiff conidiophores, 2–4 mm. in length, carry a black head, 250–330 μ thick, the dark, spherical globule measuring 58–86 μ in diameter. The primary (septate) sterigmata measured up to 100 μ in length (mean 85 μ), the diameter being 7–20 μ, whilst the secondaries measure 10–11 μ by about 3.5 μ. Here also the diameter of the dark, spherical, hairy conidia is about 4 μ. The dimensions of the heads and sterigmata are considerably larger, it is true, but the fungus ought to be cultivated for comparison with *A. niger*.

In 1896 MacAlpine (I.) described a black *Aspergillus* (*Sterigmatocystis pulverulenta*) found in all parts of *Phaseolus vulgaris*, L., which furnishes dark, spherical warty conidia, 4 μ in diameter, greatly resembling Lindau's fungus in dimensions. Culture experiments with all these species, for the purpose of observing their mutual relations, are highly desirable, it being essential to know how the form and dimensions of such fungi turn out under controllable conditions.

The fungus growing on the aged fruit of *Welwitschia mirabilis* and known as

Aspergillus Welwitschiæ (Bresadola), P. Hennings (formerly termed *Ustilago W.* by Bresadola), is also an ordinary *A. niger*, as has already been admitted by Hennings in a private communication on the subject. The same remark may also apply to the *A. ustilago* discovered by Beck in the fruit buds of *Phyllanthus Emblica* (East Indies), as well as to many others. Of course it is not impossible that other, very similar, brownish black *Sterigmatocystes* may exist—*see*, for example, P. Lindner (XXXIII.), who briefly mentions two unnamed forms of this type. The *A. atropurpureus* discovered by Zimmermann (I.) on rotting coffee berries at Buitenzorg is similar in all respects, except that the conidia are larger, being 6–8 μ in diameter. Up to the present, the conidia of *A. niger* have not been observed to share the fluctuation dimensions of the conidiophores.

Forms that are otherwise identical with *A. niger*, but differ in the slower and less abundant formation of conidia, should hardly be classed as separate species ("small species"), as was done by Costantin and Lucet (I.) in the case of *Sterigmatocystis pseudonigra*, this method leading to confusion in many respects. Gasperini (I.) found on gall-nuts, apple kernels, and solutions of tannic and citric acids, a species, *A. violaceofuscus*, which produced conidia measuring 3–3·5 by 5–6.5 μ, though recalling *A. niger* in habitat and other features.

Aspergillus candidus L., Wehmer (XVII.), occurs preferably on old, decayed vegetables of various kinds (mouldy *Pumpernickel*, rotten cucumbers, rotten grapes on the vine, spoiled cabbage-broth, mouldy cotton-seed meal, and mouldy grain), as well as on putrescent urine, old cheese, &c. The ordinarily sluggish growth of the cultures on the usual substrata also indicates that its food requirements are rather peculiar; and it seems to prefer an alkaline reaction of the medium. Probably several of the white species described in the literature will have to be amalgamated with this one; but at present it is impossible to say if it is identical with Link's old species. The surface, which is perfectly snow-white, turning creamy in old cultures, and even brown in those on wort gelatin, exhibits two forms of conidiophore: one with spherical globule and branched sterigmata, corresponding exactly with those of *A. niger* (*see* 9 in Fig. 163), whilst the other is much simpler and smaller, the sterigmata being unbranched. The conidia are mostly ellipsoidal, smooth or covered with fine dots, and 2.5–4 μ in diameter. The *A. albus*, described by Wilhelm (I.) in 1877, with its spherical globule and branched sterigmata—differing from the specimens found nearly always on spoilt barley by P. Lindner (XXXIII.)—probably corresponds to the larger form. A critical investigation of the white species, on the basis of culture experiments, is highly desirable, this group being at present in a chaotic state, unless one is content with imperfect descriptions and artificial specific names (*see* the forms arranged by Wehmer (XVII.) and Lindau (I.)).

Aspergillus nidulans (= *Sterigmatocystis nidulans*, Eidam). This species, which is pathogenic when injected into the blood (optimum temperature about 40° C.!), and is also sometimes found in the human ear, was first discovered by Eidam, in 1883, in a humble-bees' nest. It is a scarce, handsome green species, and is rendered interesting by its sclerotia, which, however, has only been observed and studied in a single instance, Eidam having failed to discover it again. In 1904 Saito (I.) had a specimen, which he did not examine further, but states that the fungus occurs in the air in Japan, associated with *A. glaucus*. The tough-skinned conidiophores (Fig. 176) on the green surface (which afterwards becomes discoloured) measure up to 0.6–0.8 mm., but are frequently only one-third to one-half that size. The branched sterigmata of the insignificant, club-shaped globule (15–20 μ thick), which recalls *A. fumigatus*, are usually confined to the upper half, and generally produce globular, smooth (or finely dotted), very small conidia (3 μ in diameter) in long chains adhering in the form of tough masses. Septation and branching of the stems (sometimes very

irregularly) seem by no means infrequent. According to Eidam's observations on the development of the ascospore, this latter is formed from two hyphæ (instead of one, as in the case of *A. glaucus*), the one developing into the tough, stratified, pseudoparenchymatous integument, whilst the other furnishes the internal tissue forming the asci. Several weeks pass before the development is complete, and the ascospore, which is then provided with tough, dark blackish red walls, is ripe. The gradually evolved, ovoid ascus, 10–11 μ in length, encloses eight smooth, lenticular spores (about 5 μ by 4 μ), provided with a longitudinal furrow and a tough purple epispore, which bursts in two during germination. Analogous to those of *A. fumigatus* and *A. Rehmii*, the sclerotia (0.2–0.3 μ in diameter) are surrounded by a shell of peculiar, yellowish hyphæ, which are distended like bubbles—this feature is absent in *A. glaucus* and *A. pseudoclavatus*. We need do no more than mention the ascosporogenic *A. pseudonidulans* (Vuill.), recognised by Vuillemin (I.) as a *Sterigmatocystis*.

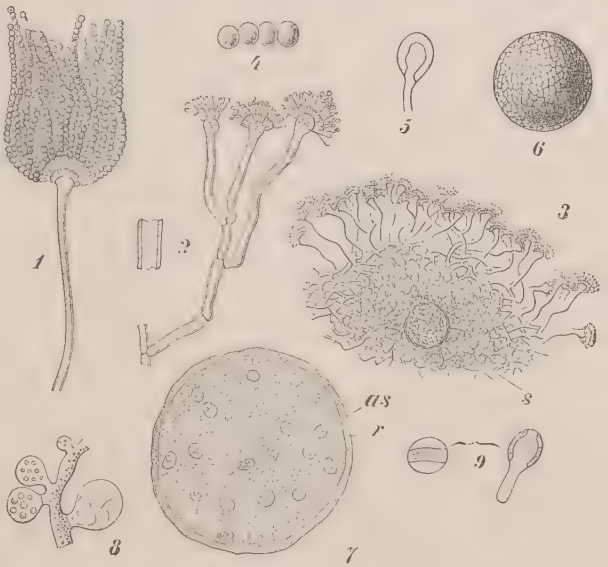


Fig. 176.—Aspergillus nidulans.

Conidiophores with branched sterigmata (1 and 2), conidia (4), ascospore with globular capsule (5), prepared separately at 6, and shown in section at 7, with asci (8), spores (9), one of them with germinating tube. Magn. of 1 and 2, 330; of 3, 120; of 4, 1000; of 6, 85; of 7, 170; of 8, 400. (*After Eidam.*)

A. Rehmii, Zukal, and *A. pseudoclavatus*, Puriewitsch, are also species with branched sterigmata. Both of them are rare and of little practical importance, but noteworthy as being among the few reported as producing ascospores, which, however, are quite different from those of *A. nidulans*.

Aspergillus Rehmii (*see* 3 in Fig. 165) was discovered on gall-nuts and decayed oak bark by Zukal (I.) in 1893. The sulphur-yellow to ochreous coat develops dwarf conidiophores (0.4–0.5 mm. high), bearing elongated ovoid globules (20 μ by 30 μ), slender sterigmata, and small globular to ellipsoidal conidia (2.5 4 μ across). The black, fragile perithecia (0.1–0.2 mm.), the skin of which is formed of a single layer of regularly disposed rows of cells, are surrounded by a compact shell formed of yellow hyphæ, which in many cases are swollen into globules. The asci, which are formed immediately, are ovoid, on short stems and rapidly become mucinous, develop elliptical, tough-skinned, smoke-grey spores, measuring 5 μ by 3.5 μ and numbering 8 in each case. The ascospores are formed by the intertwining and fusion of morphologically uniform hyphæ. This species may be regarded as doubtful.

Aspergillus pseudoclavatus, Puriewitsch, agrees, in the structure of the conidia (up to the branched sterigmata) entirely with that of *A. clavatus*. The globule measures 260–300 μ by 60–70 μ; and the greyish green, ellipsoidal conidia,

measuring 3·5–4 μ by 2·5–3 μ, are identical in size and shape with those of that species. The small naked globular perithecia, which measure 60–70 μ in diameter, and are provided with a wall formed of a single layer of cells, enclose only 6–7 asci, each with 8 colourless spores. The perithecium is apparently developed from two hyphæ. The optimum temperature of this species, which was discovered on old yeast cultures by Puriewitsch (IV.) in 1899, is about 25° C.

Of the other best known *Sterigmatocystes* we need mention only the following: the brownish yellow *A. sulfureus*, Fresenius (on bark); *A. ochraceus*, Wilhelm (on bread and damp portions of plants), which develops sclerotia abundantly, but no asci; the green *A. elegans*, Gasperini (on decaying lemons); *A. variabilis*, Gasperini (on decaying fruit), with both simple and branched sterigmata. Allied to these are a number of more or less doubtful or imperfectly described species, found chiefly on vegetables, and included in Saccardo's list (IV.), and also critically sifted by Wehmer (XVII.). The *A. ochraceus*, described and closely examined by Wilhelm (I.), produces a large number of brown, nodular sclerotia, formed by the intertwining and fusion of ordinary hyphæ (as in the case of *A. niger*), but not developing asci. This seems to be identical with the *A. auricomus* of Guéguen (I.). Recently, Vuillemin and Mirsky (I.) described *A. versicolor* (*Sterigmatocystis v.*), and Guéguen (III.) an *A. syncephalis*. The former is of interest, owing to the variable colour of its cultures, and has latterly been repeatedly investigated by Mirsky (I.), Vuillemin (II.), Friedel (II.) and by Coupin and Friedel (I.). The conidiophores are similar to those of *A. niger*, but the optimum temperature of growth is much lower, and no development takes place at all at 37°–39° C. The mycelium is a rusty brown, and no perithecia or sclerotia are formed. The red pigment, which is soluble in alcohol, is developed in the green cultures exclusively. The fungus also appears in a reddish form (with pink conidia), which, however, reverts to green sooner or later. No morphological details seem to have been published in connection with this species.

The following are probably synonyms, or at all events unrecognisable, owing to imperfect description, though they have found a place in the more recent literature: *A. luteus* (v. Tiegh.); *A. flavescens*, Wred. (same as *A. flavus*, Link), *A. nigricans*, Wred. (also Cooke); *A. nigrescens*, Rob. (both probably *A. niger*); *A. terricola*, March. (probably *A. flavus?*); *A. griseus*, Link, (*A. fumigatus?*); *Eurotium malignum*, Lindt (probably *A. fumigatus*, Fres. ?); *A. quininæ*, Heim; and *A. subfuscus*, Johan-Olsen (*A. flavus?*). In any case, the only way to justify these names is by describing the fungi in such a manner as to admit of their identification; otherwise the reader is left in doubt. Even the scientific literature does not, unfortunately, always give the correct names; Green (I.), for example, referring to the well-known *Aspergillus oryzæ* as "*Eurotium oryzæ*."

§ 286.—The Genus Penicillium.

The *Penicillium* group, which, though less important, both scientifically and practically, than Aspergillus, possesses considerable interest on account of its characteristic conidiophores, comprises a number of species which are more or less analogous, and are chiefly met with in practice as producing mould on vegetables, inhabiting cheese, or acting as putrefactive fungi.

The microscopically small and delicate conidiophore, which is morphologically on a far lower stage of development than that of *Aspergillus*, differs from an ordinary vegetative hypha solely in the method of branching and the fairly upright growth, being inappreciably thicker, and just as thin-skinned and septate as the latter. The slender sterigmata, which are developed successively in

whorls or tufts, occupy the undistended ends of main and lateral branchings, which grow to an almost uniform height and are mostly upright. The lateral branches are usually two to four in number, sometimes alternate and sometimes in whorls, a considerable amount of variation being, however, observed in the structure of the conidiophores belonging to the same species. This method of

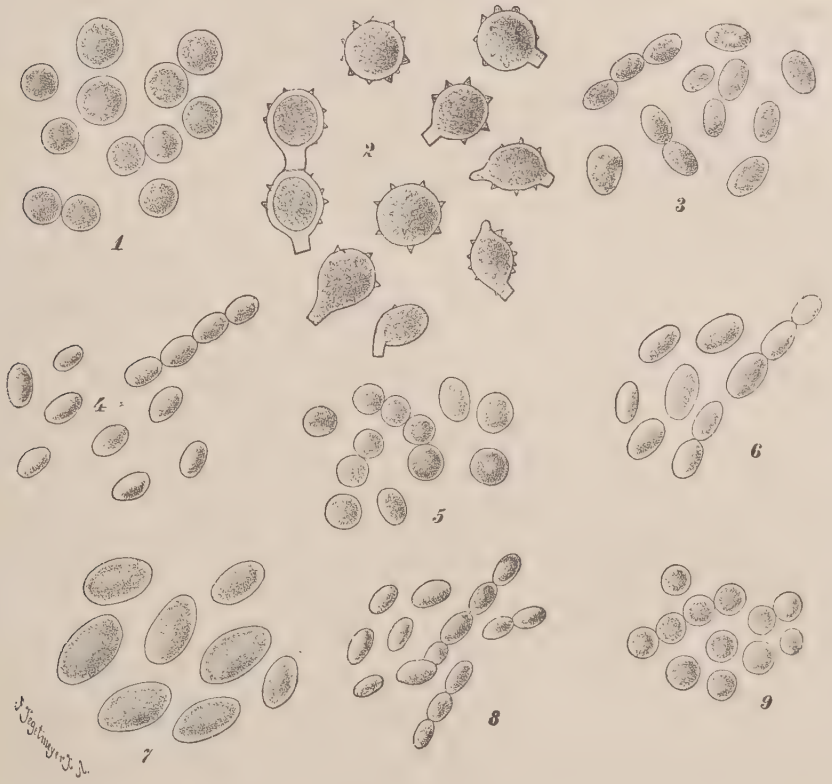


FIG. 177.—Conidia of various species of Penicillium, all drawn to the same scale. (Magn. about 1200).

1. P. Camembert (conidia 3.1–4.5 μ in diameter); 2. P. brevicaule (7–10 μ by 5.7–6.8 μ); 3. P. purpurogenum (2.8–3.3 μ by 2 μ); 4. P. claviforme (3 μ by 2 μ); 5. P. rubrum (2.8–3.5 μ in diameter); 6. P. italicum (4–5 μ by 2–3 μ); 7. P. olivaceum (6–10 μ by 4–6 μ); 8. P. luteum (2.3–3 μ by 1.4–2 μ); 9. P. glaucum (2.5–3 μ in diameter).
Measured on growths from pure cultures on wort gelatin. (*Original.*)

branching produces the characteristic brush shape of the conidiophores. The sterigmata usually diverge in a very appreciable manner, and vary in number from two to ten, their relative length (referred to the head) and pointed shape varying with the species, but being generally constant in one and the same species. The conidia of the commoner species ("*P. glaucum*," *P. luteum*, *P. italicum*) are globular to ellipsoidal, mostly glabrous, thin-walled, and almost colourless when taken singly, but producing the characteristic colour of the growth when observed in the mass. They are mostly small (about 2.5–5 μ in longest diameter), but in isolated instances (*P. oliraceum*) may grow to a length of 10 μ. The younger members of the long chains are often appreciably smaller,

of different shape, and firmly attached together throughout, only becoming loosened after they have grown considerably.

The germination of *Penicillium* conidia, observed by E. Loew (I.), presents no special features.

Several species are distinguished by a tendency to the formation of a coremium (*see* vol. ii., p. 17), which in some cases occurs spasmodically, being apparently dependent on circumstances (*P. luteum*, *P. glaucum*), whilst in others it is a regular feature (*P. granulatum*, *P. claviforme*) under nearly all conditions. The arboriform coremia of *P. luteum* are noticeable on account of their size (up to 1 cm. in height) and ornamental appearance. Those of *P. claviforme*—described by Bainier (I.)—differ from the others in that handsome isaria-like clubs are formed, which initially appear white, but afterwards turn green on the top from the presence of conidiophores. This fungus, the surface of which remains sterile, the conidia being produced solely on the clavate stroma, about 1 cm. in height, should more properly be grouped with *Isaria*.

Ascospores, in the form of small, coloured globular nodules of highly diversified character, have been found in four to five species. In the case of *P. luteum*, *P. aureum*, *P. insigne* (?) they are soft-skinned, with continuous development. In the last two the skin is pseudoparenchymatous, whereas in the first one they consist of hyphæ, somewhat loosely connected at first, but afterwards coherent. Tough sclerotia, forming asci after a lengthy period of rest (intermittent development), are formed by *P. glaucum*, Brefeld, and similar though sterile forms are found in *P. italicum*. The perithecia of *P. aureum* (which seems closely allied to *P. luteum*) are said by van Tieghem (IV.) to possess a yellow mycelial integument as well. Morini (I.) reported the occurrence of perithecia in *P. candidum*, Link, but gave no further particulars regarding the form of the conidia; and, since pictorial representations are lacking, the question must be left undecided. The same also applies to *P. Wortmanni*, Klöcker (*see* p. 255, vol. ii.). More detailed particulars on the progress of development are scarce and also contradictory in many respects. The ascospores are ellipsoidal, with the epispore tough, glabrous (*P. aureum*), warty (*P. insigne*, *P. Wortmanni*), or thickened in ridges (*P. glaucum*, Bref., *P. luteum*, Zuk.), the epispore being with (*P. glaucum*, Bref.) or without (*P. luteum*, Zuk.) a longitudinal furrow.

Ascospores not having been detected in a large number of species, the criteria of differentiation of the various species include the colour of the vegetation (mostly green in all shades from bluish to brownish, though yellowish, white, and brown species are known); the branching of the conidiophores and the size and shape of the conidia, together with other, slighter characteristics, especially those of a physiological nature, such as pigmentation, energy of growth, gelatin liquefaction, acid production, food-stuff requirements in respect of the various sources of carbon, nitrogen, &c. Except in the case of the collective species "*P. glaucum*," little was known until recently of their requirements as to temperature. No mention has yet been made of species that thrive at blood-heat, and the maximum seems to be lower than 37° C. Stoll (I.) quite recently published certain observations in this connection with regard to six species more closely examined, morphologically and in cultures, by him, from which it appears that only *P. purpurogenum* and *P. rubrum* thrive best at higher temperatures (30° C. in the one case and 30°–35° C. in the other), the optimum temperature for the remainder being below 30° C., viz., *P. italicum*, 25° C.; *P. olivaceum*, 23°–25° C.; "*P. glaucum*" and *P. brevicaule*, 20°–23° C. The colour of old herbages, especially when grown under uncontrollable conditions, is, of course, useless as a means of differentiation, and probably a number of grey, brown, and dark-coloured species mentioned in the older literature owe their existence to these fictitious differences. As in the case of *Aspergillus*, the colour is frequently dependent on the character

of the substratum, an alkaline reaction of the latter appearing to cause the green to turn greyish brown.

In the present unsatisfactory state of knowledge on the *Penicillium* group, the number of species taking part in the ripening of cheese, and the production of mould and decay in fruit, is about six or seven, though the list will probably be increased to some extent in time. A noteworthy fact, in comparison with *Aspergillus*, is the absence of any species pathogenic to animals, or of technical value outside Europe; at least, the few that are said to be pathogenic, inhabiting mucous membrane and animal substrata, have a very doubtful existence as distinct species (*P. quadrifidum*, Salisbury; *P. pruriosum*, Salisb., &c.), and there is a wide field open for subsequent research. A so-called *P. minimum*, found by Siebenmann (I.), in the ear of a patient, needs further explanation. F. Dierkx (I.) in a recent preliminary communication, gave no less than twenty-two new species (almost completely ignoring those already known), but gave no illustrations or sufficient description of them. Moreover, the habitat of the newly found "species" being unstated, it is difficult to accept them as genuine, and the matter is not advanced at all by this communication, despite the accuracy of the principles laid down by that author, who lays stress, *inter alia*, on the necessity for culture experiments for describing a species. A publication by Stoll (I.), shortly before the completion of the present manuscript, adds to our knowledge of the *Penicillium* group, by detailing a series of observations on comparative cultures of several species. Further elucidation of this difficult subject may be anticipated from the investigations of Thom (I.), which up to the present have only been outlined.

It hardly needs emphasising that the form and size of the conidia are constant for one and the same species, and that reports on the transformation of growths with ellipsoidal conidia into such as produce globular conidia—as described by Guéguen (II.)—must be regarded very critically.

Nothing certain can be stated with regard to the number of species in existence. Undoubtedly a large proportion of the fifty odd alleged species included in Saccardo's list (IV.) will have to be deleted, especially since the older descriptions are insufficient for identification. Scarcely one-half of the above number have been clearly characterised, and only a portion of this moiety can be regarded as authentically established. Nearly half the thirty-two species counted by Lindau (II.) are unrecognisable or doubtful, the old descriptions given by Preuss, Corda, and Bonorden being insufficient as a starting-point, it having been customary at that time to simply describe, without troubling about the previous work of others, in a way that is quite incommensurate with modern requirements. The greatest confusion exists at present with reference to the fungus termed "*Penicillium glaucum*," of which there appear to be several closely allied species included under this collective name in the literature. In fact we are only on the threshold of real knowledge in connection with the *Penicillium* group. A summary of the *Penicillium* species is given below, the technically important members, that have been more accurately described and are dealt with fully later on, being marked with a *. Fuller particulars are set forth by Saccardo (IV.) and by Lindau (II.).

Summary of Penicillium Species.

1. Conidial herbage, green:
   P. glaucum * (Link ?) Bref., Sclerotia with subsequent formation of asci; *P. italicum*.* Wehmer, sterile sclerotia; *P. olivaceum*,* Wehmer; *P. luteum*, Zukal, soft-skinned ascospores; *P. rubrum*, Stoll; *P. purpurogenum*, Stoll; *P. aureum*, Corda, soft-skinned ascospores (perithecia); *P. radiatum*, P. Lindner (sclerotia ?); *P. Wortmanni*, Klöcker, soft-skinned ascospores (like *P. aureum* and *P. luteum*); *P.*

Duclauxii, Delacroix (*P. luteum?*), *P. Camembert*,* ad int. (*see also* under 4); *P. Roquefort*, ad int. ; *P. claviforme*, Bain. ; *P. granulatum*, Bain.

2. Conidial herbage yellowish to brownish or brown :
   P. brevicaule,* Sacc.
3. Conidial herbage reddish to red :
   P. roseum, Lk. (?).
4. Conidial herbage white to light grey :
   P. candidum, Lk., sclerotium with formation of asci ; *P. Camembert*,* a.i. (herbage temporarily a faint green) ; [*P. insigne* (Winter), Schröter, with formation of perithecia (= *Gliocladium penicilloides*)].

As already mentioned, it hardly seems advisable to subdivide this morphological genus at present, even in cases where the course of development of the separate species seems to assign them to different places in the system. Thus, the great difference between the ascospores of *P. glaucum*, Bref., and *P. luteum*, Zuk., involves their allocation to two different genera, whilst any species exhibiting true perithecia would have to be placed in a third genus, leaving the numerous unallotted species to rank as "fungi imperfecti" in a fourth group. For this, however, it is preferable to wait until the species are better known, retaining in the meantime the genus *Penicillium* as a group of species classed together by their conidiophores.

§ 287. The Species of the Genus Penicillium.

The species most frequently encountered, generally of technical or pathological importance, will be dealt with first, chief among them being *Penicillium glaucum* (Link ?), Brefeld ; (*Penic. crustaceum*, Fries ?). The *P. glaucum*, Link, of the literature is evidently a collective name for a series of closely allied green species, a thorough examination of which is highly desirable. The colour of the growths, the branching of the conidiophores and the size and shape of the conidia are very similar in all. If it be desired to preserve this specific name from extinction—it is impossible now to say what Linné, Link, Fries and others had before them—it would be most appropriately bestowed on the species more closely studied by Brefeld (II.), which produced very small, spherical conidia (2.5 μ in diameter), and sclerotia, all differing from this form being named afresh. That a large number of these do exist is sufficiently demonstrated by the recent investigations of Thom (II.). In these circumstances it is difficult to assign to any particular form the numerous reports in the literature relative to the occurrence and action of the collective species "*P. glaucum*." For instance, the "*P. glaucum*" concerned in the ripening of cheese can apparently be subdivided into several distinct species, readily distinguishable macroscopically, in pure cultures, from each other and from "*P. glaucum*." Two of them, in fact, are described a little later on, under the names *P. Roquefort* and *P. Camembert*. Differing from these again are the species furnishing round spores, and appearing as the cause of decay in ripe fruits, but not yet closely examined. The classification of the so-called *P. glaucum*, Link, species of green mould observed on hops, shed-ripe tobacco, in the leather manufacturing process, and in vinous fermentation (the cause of mouldy flavour in wines) will have to be postponed until they have been more closely compared by the customary mycological methods. This group is comparatively easy to differentiate from the species producing elongated spores (*P. luteum*, *P. italicum*, *P. olivaceum*), and also from the macroscopically similar members of other genera (*Citromyces* species, *Aspergillus fumigatus*), distinguishable at once by the structure of their conidiophores, though confusion has probably occurred in the literature from the grouping of all green moulds as *P. glaucum*, Link. Nothing, in fact, is so

deceptive as the exactly similar green shade common to the growths of a large number of species, most of the *Penicillium* group being of this colour.

The *Penicillium glaucum* of BREFELD (IV.) exhibits the following characteristics (*see* Fig. 178). The conidia are globular, smooth, 2.5 μ thick, and occur as long coherent chains on pointed cylindrical sterigmata, measuring about 5–13 μ long and 3–4 μ thick. The branching of the conidiophores varies considerably (*see* Fig.), and these organs measure 200–400 μ in length, each twig being crowned with a tuft of (up to 12) sterigmata, which are usually shorter than their bearing cells. The colour of the herbage and vegetation is pale, or dark green, becoming discoloured with age; structure compact, not woolly, conidia abundant. Under certain conditions, though not very regularly, the species forms small, hard, spherical to nodular sclerotia, resembling grains of sand in size (0.1–0.8 mm. in diameter), and gradually forming asci, after a period of repose, by resorption of the tough central tissue. The closely crowded, globular to ellipsoidal asci (12–15 by 8–10 μ) fall apart eventually, so that when ripe (after about 7–8 months) the interior of the organ, which is surrounded by a stratified skin (2–3 layers), is full of free, pale yellow, ellipsoidal spores (5–6 by 4–4.5 μ). In germination, the individual spores, which have a longitudinal groove and 3–4 transverse ribs, throw off the two halves of the epispore. According to Brefeld's earlier reports, the sclerotium is developed from two special hyphae (the ascogonium and pollinodium) by a kind of fructification process, the asci being then formed as lateral shoots from the ascogonium. ZUKAL (II.), on the other hand, describes the

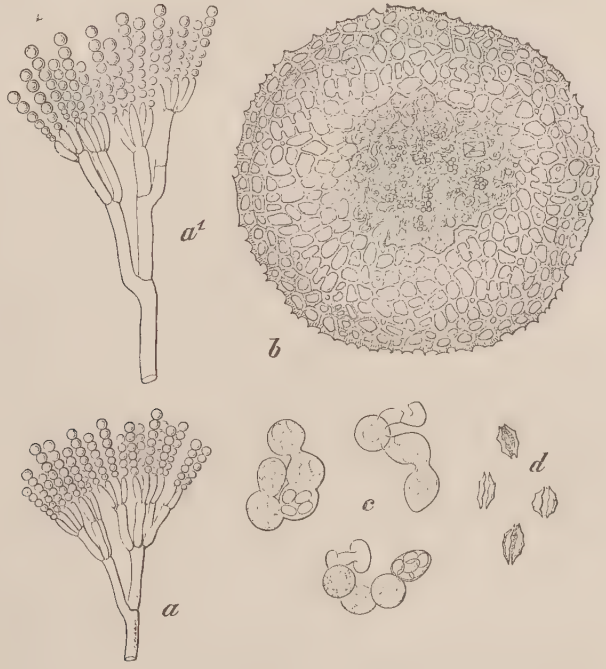


FIG. 178.—Penicillium glaucum.

Conidiophores exhibiting diversified branchings (*a*, *a'*); ascospore with ripening asci (*b*); isolated ascus in course of sporogenation (*c*); spores viewed laterally (*d*). Magn. of *a*, 315; of *b*, 150; of *c*, 630; of *d*, 800. (*After Brefeld.*)

formation of the sclerotium as resulting from the fusion of two equivalent, simple, vegetative hyphæ, and has observed the asci developing from the filaments growing out from the wall of the hollow sclerotium into the interior cavity of same.

It seems evident, from the varying dimensions of the conidia, and especially from the reports on the limits of temperature, that the *P. glaucum* described by various authors was not always one and the same fungus. Only the dimensions given for the conidia by SCHRÖTER (I.), namely, 2–3 μ, and by WEHMER (XXIII.), namely, 3 μ, agree closely with the figures given by BREFELD (II.), SACCARDO (III.) reporting them as measuring 4 μ, Lindau 3–4 μ (globular or ellipsoidal conidia), and STOLL (I.) 3.8–4.3 μ. The latter worker in particular seems to have had before him a very different form, with round spores, since it thrives as well at 37° C. as at 8° C., the maximum temperature being even above 40° C., whereas, as a matter of fact, most of the forms of this class die off completely at 37° C. Others have found the minimum and maximum temperatures for *P. glaucum* as 1.5°–2° C. and 33°–35° C. respectively. If Grawitz formerly habituated the fungus to temperatures of 38°–40° C., and then made successful inoculations on animals, he could hardly have been working with a form of *P. glaucum*. The form grown by STOLL (I.) gave a pure white instead of green vegetation on agar-agar after several reinoculations, thus producing a white form, analogous to *P. candidum*, Link, which, however, reverted to the green form and produced the normal conidial pigment, when transferred to ordinary media. Actual proof is lacking in support of GUÉGUEN's assumption (II.) that the species known as *P. glaucum* varies considerably in the form of its conidia, and that a form with round spores can pass over into a form with elongated spores. This is, moreover, very unlikely, and the probable explanation is that similar but really different species were present in the mixture, since the shape and size of the ripe conidia have hitherto been found very constant, and there is no really accurate experience of any variability in the morphological characteristics of one of these forms, if we except the irregular branching of the conidiophores. Reports on sclerotia are given by WINTER (IV.) and GUÉGUEN (II.), and on the formation of coremia by BREFELD (II.) and HENNINGS (II.).

The two following species can be differentiated from Brefeld's fungus on the ground of THOM's investigations (II.), and also differ from each other in form and culture. Thom named them "Roquefort mould" and "Camembert mould" respectively; but, in the absence of any specific names, they may be provisionally termed *P. Roquefort* and *P. Camembert*.

Penicillium Roquefort, THOM's (II.) Roquefort mould, hitherto generally called *P. glaucum*, Link, differs clearly from Brefeld's *P. glaucum* by the size of its conidia, which are about twice as large as those of the latter. The position of this species, which is of regular occurrence, in the conidiophore-bearing stage, in the green veins of ripening Roquefort cheese, is left an open question by Thom. The conidiophore is 200–300 μ high and 4 μ thick, the average height of the conidia heads is 90–120 μ; and the branchings are arranged in irregular whorls, carrying sterigmata 9–11 μ long and 2.5 μ across. The conidia are bluish green, mostly spherical, smooth and large, being 4–5 μ in diameter. The colour of the vegetation is dark green, afterwards turning to a dirty brown, the underside being yellowish white. No ascospores have been detected. Only a slight liquefactive action was exerted on sugar gelatin; and red litmus was rapidly turned blue. The germination of the conidia and development proceed rapidly, an abundant mycelium, with conidia, being frequently produced within thirty-six hours. This rapid growth distinguishes the species from *P. Camembert*, and the conidia are less sensitive to drought, sometimes retaining their germinating power for months. According to CONN, THOM, BOSWORTH, STOCKING and

Issajeff (I.), a bitter taste is imparted to the cheese. Thom states that the species is characteristic for Roquefort cheese, though it occurs on many other substrata and appears to be distributed everywhere.

Penicillium Camembert, the Camembert mould of Thom (II.), is a distinct species which plays a constant part in the ripening of Camembert cheese. Conn, Thom, Bosworth, Stocking and Issajeff (I.) call it simply Camembert fungus, leaving the species undefined owing to lack of sufficient description. Thom (II.) has also recently described it more closely under the name Camembert mould (*P. album*, Epstein ?), and it is probably identical with Roger's (I.) *P. candidum* from Brie cheese (1898), and Epstein's (I.) *P. album* from Camembert cheese (1902), which have not been morphologically described. In re-naming the species, *P. Rogeri* would perhaps be more applicable than *P. Epsteini*, suggested by Lindau (II.). Thom states that the vegetation is white at first and decidedly woolly (not smooth !), the colour gradually changing to pale greyish green and afterwards greyish white. The conidiophores are 300–800 μ long and 3–4 μ thick, the conidia heads are up to 175 μ in length and slightly branched. Sterigmata are not numerous (8–11 μ by 2.4–3 μ), the ripe conidia are globular (cylindrical to ellipsoidal while young), bluish green, large, 4.5–5.5 μ thick and smooth. The mycelial threads are about 5 μ in diameter. Conidia are formed on the free surface only, not in cavities in the substratum. Sugar gelatin is liquefied under the colonies only, litmus being turned red at first, but quickly blue again. Cheese inoculated with this species is covered over in a week with a woolly white mycelium. The fungus does not seem to occur in the open ; and Thom regards it as a typical dairy species which will not grow under other conditions. Even as an infection, it rarely occurs on other kinds of cheese. The conidia lose their power of germination if kept perfectly dry for a few weeks. It is said to peptonise milk without any previous coagulation, and to assume a faint yellow colour, without emitting the pungent ammoniacal smell produced by "*P. glaucum*" (Roquefort-P.). The slight acidity set up in the substratum at first, soon disappears. In pure cultures, the species can be distinguished from the two preceding ones at a glance.

Penicillium luteum, Zukal, forms green vegetations, distinguishable from the other species by their faintly brownish (olive) tone. The sterile mycelia are often characterised by a bright lemon-yellow coloration, which is afterwards masked by the incipient conidia, and is then only visible at the edges, if at all. It differs from the ordinary species by its small ellipsoidal conidia and very long sterigmata. It frequently occurs on substrata that are prone to mould (skins, fruits, paste, &c.), especially preferring those of an acid character (lemons), and according to Behrens (IX.), it causes fruit to rot, by producing poisonous substances. On account of its tenacity and rapid growth, it is a source of trouble in places where it has once found a lodgment, and when infecting other fungi it frequently kills them off rapidly. According to Wehmer (XXXIV.), this is especially the case with *Citromyces*, on the vegetations of which it produces brown, slippery, dead patches which rapidly spread outwards. The tenacity of life on the part of the conidia is, however, very slight; and as a rule they all die off in one or two years. The delicate conidiophores (Fig. 179), more closely examined by Wehmer (XX.), branch like the two preceding species, but are characterised by a tendency to form whorls, so that the main filaments usually exhibit only a single whorl of 2–4 branches of the first order bearing tufts of sterigmata, though a variety of deviations occur. The sterigmata (17 by 2.1 μ) are more pointed, and longer (in comparison with the head) than in most other species, the conidia decidedly elongated (ellipsoidal), very small (2.3–3 by 1.4–2 μ)—*see also* 8, Fig. 177—smooth, delicate, hanging together firmly in long chains, dull grey in colour, but greenish grey when heaped up.

According to Wehmer (XXI.) coremia are frequently developed, occasionally extensive, very handsome, and up to 1 cm. in height. The ascospores, which were first observed by Zukal (III.), and afterwards more particularly described by Wehmer (XX.), are usually abundant, occurring on the surface of the vegetation as lemon to golden yellow, thin-skinned bodies, more or less spherical, 1–2 mm. in diameter, turning dark orange with age, and ultimately becoming discoloured. The integument, which is about 100 μ thick, formed of loosely woven hyphæ and of a golden yellow (afterwards brownish red) colour, encloses a colourless network of filaments with embedded nests of ellipsoidal asci (measuring 9–11 by 6–8 μ), each of which contains 4–8 (average 5) barrel-shaped, tough-walled spores (4–5 by 2–8 μ). In contrast with those of *P. glaucum*, Brefeld, these spores have no longitudinal furrow, but are provided with 3–4 delicate transverse fillets, which do not eject the epispore in two halves during germination, but allow the contents to escape through fine cracks and form a voluminous secondary spore, which then develops. In a few weeks the integument of the fruit becomes very brittle, and encloses a pale yellow dusty mass of liberated spores. These ascospores, which at first consist of loosely intertwined bundles of hyphæ with separated groups of asci, would undoubtedly justify the classification of the species outside the *Aspergillaceæ*, the fructification being similar to that of *Gymnoascus* and differing completely from *P. glaucum*, Brefeld. The bright yellow colour of the young mycelium and fruit case of *P. luteum* is due to yellow granules (a pigment soluble in alcohol and classed by Zukal as a fungus acid), abundantly secreted by the hyphæ and forming a dense coating upon them. These are lacking in *P. glaucum*, and are not invariably met with in *P. luteum*. The fungus readily acidifies saccharine nutrient media by the formation of free citric acid.

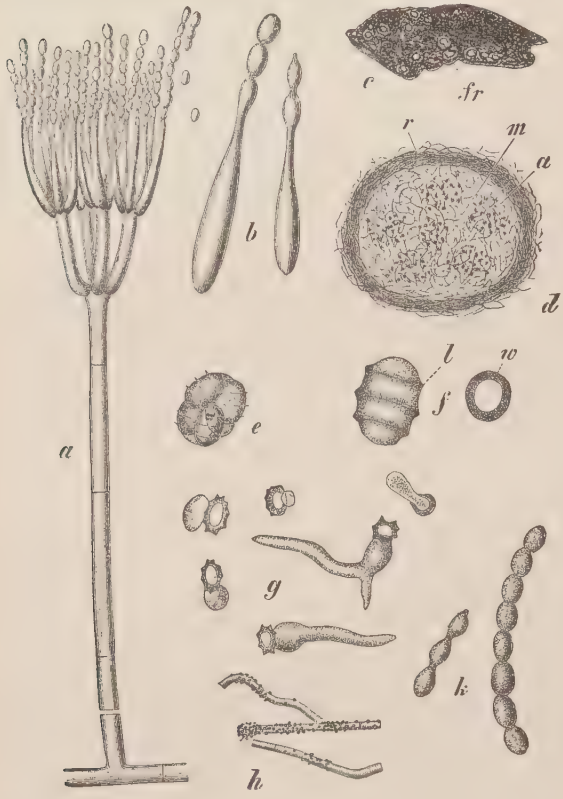


Fig. 179.—Penicillium luteum.

Typical conidiophore (*a*), sterigmata (*b*), and conidia (*k*); *c*, ascospores on the vegetation (nat. size); *d*, section of ascospore with medulla (*m*), skin (*r*) and groups of asci (*a*); *e*, free ascus; *f*, spores viewed from the side and in section, showing barrel-hoop fillets; *g*, germination of ascospore; *h*, hyphæ with yellow granules. Magn. of *a*, 1000; of *b*, 2000; of *d*, 15; of *e*, 1200; of *f*, 2400; of *g*, 900; of *h*, 500; of *k*, 2000. (*After Wehmer.*)

Penicillium italicum, Wehmer, is a mould which, according to Wehmer

(XXIX.) is found only on certain substrata (pine-apples, lemons, oranges, and similar southern fruits), and differs from those already described by the bluish grey shade of the green surface. The structure of the conidiophores (*see* Fig. 180) corresponds with that of *P. glaucum*, Brefeld, but the conidia are ellipsoidal instead of spherical. This fungus, which is very commonly imported with the fruits in question, is the cause of extensive putrefaction, for instance, in the case of ripe pine-apples, the entire contents of the closed cases being sometimes destroyed in this way during transport. The rapid spread of the mould on the

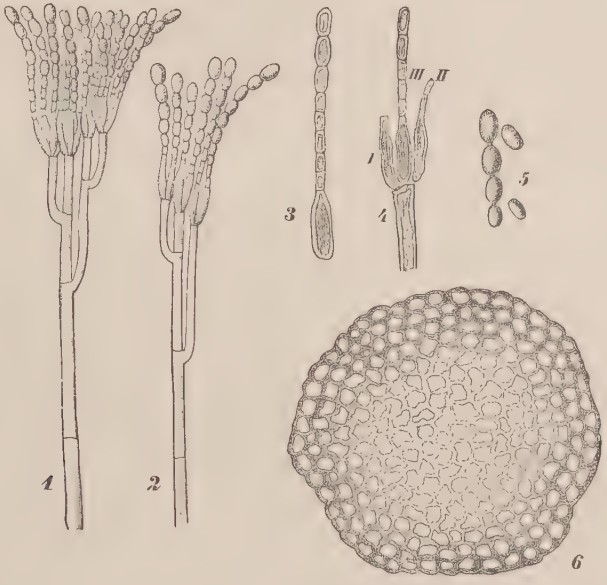


FIG. 180.—Penicillium italicum.

Conidiophores (1, 2), sterigmata (3, 4), and conidia (5). Section through an old sclerotium (6), the coloured strata of the rind shaded more darkly. Approx. magn. of 1–2, 400; of 3–4, 600; of 5, 700; of 6, 90. (*After Wehmer.*)

surface is accompanied by an equally rapid penetration of the flesh of the fruit, which is spoiled in consequence. The delicate, colourless conidiophores, which are only of the thickness of hyphæ and about 250 μ long, carry 2–3 upright branches, arranged at unequal heighths and provided with a tuft of (2–6) sterigmata, like the main stem, but not always at the same height. The delicate ellipsoidal conidia, extending in long chains from the slender, tapering sterigmata (measuring about 10 by 3 μ), hang together at first like the cells of a closely septated hypha, but afterwards become more rounded, increasing considerably in volume and becoming looser, their fairly uniform dimensions then being about 4–5 by 3 μ, though sometimes as much as 6.1 by 4 μ. Individually almost colourless, they give rise to the characteristic shade of the vegetation when closely packed together. The fungus develops abundant sclerotia, differing but slightly in size, form, and tough structure from those of *P. glaucum*, Brefeld. They form small, smooth, brown, fairly uniform, hard, brittle globules, about 300 μ in diameter, either enveloped in mycelium or bare, and are easily separated at any time by rubbing the vegetative coating between the fingers. Since, up to now, all experiments with a view to the development of ascospores have failed, the sclerotia must, for the time being, be regarded as sterile, in which respect they are

on a par with those of *Aspergillus flavus*, *A. ochraceus*, and *A. niger*. Nothing definite is yet known as to their life history. The fungus is readily cultivated on the usual mycological substrata, and when grown on sugar solutions containing inorganic salts, forms tough, closely matted coatings, colourless below and pale to greyish green above, a large number of conidia being produced. According to Stoll (I.), the optimum temperature is 25° C., and the minimum 10° C. The liquefaction of the gelatin is effected very slowly, or may be entirely absent (according to the composition).

Penicillium olivaceum, Wehmer, is said by Wehmer (XXIII.) to occur, like the foregoing species, almost exclusively as a putrefactive organism on southern fruits, the two being sometimes found together. It is also occasionally met with on European fruit, Zschokke (I.) having found it as the cause of gradual putrefaction on pears. His description, however, might apply to *P. luteum*, which has also been found on fruit by Behrens (IX.). The colour of the vegetation is an olive-green, like that of *P. luteum*, but brighter, and lacking the yellow granules excreted by the sterile hyphæ of the latter. The conidiophores are handsome, but scarcely visible to the unassisted eye, so that the herbage does not appear filamentous. The conidiophores (Fig. 181), which measure up to about 200 μ in length, are less regular in structure than those of the preceding three species, there being no well-defined average system of branching. The branches are 1–3 in number, each carrying a few (2–3) sterigmata, about 14 by 3 μ. The conidia are ellipsoidal, like those of *P. italicum*, but much larger, averaging 6–7 by 4 μ, though sometimes attaining 10 by 6 μ, and joined together in chains which readily fall to pieces, only the younger and much smaller ones ($\frac{1}{3}$–$\frac{1}{2}$ μ) being firmly connected. The conidia are therefore twice as large as those of the preceding species. No fructification has yet been observed. On artificial substrata, the species forms a yellow-green coating of mould. The optimum temperature of growth is 23°–25° C., the minimum being about 10° C. The liquefactive action on gelatin is very slight. Further particulars and observations on the cultivation of the species are given by Stoll (I.).

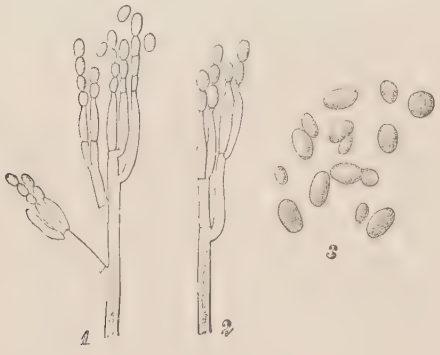


Fig. 181.—Penicillium olivaceum.

Conidiophores and conidia. Approx. magn. of 1–2, 400; of 3, 502. (*After Wehmer.*)

Penicillium brevicaule, Saccardo, observed by Saccardo (III.) with other moulds on decayed paper, has been recommended by Gosio (II. and III.) as a reagent for the detection of arsenic, since, when grown in media containing traces of that substance, it forms the pungent compound, diethylarsine (*see* vol. ii. p. 37). The morphological and biological conditions have recently been more closely described by Stoll (I.), though not exhaustively so. The growths are brownish yellow to brown in colour, according to the age and substratum. The conidiophores (Fig. 182) are delicate and small, irregularly branched, and usually with but few twigs and sterigmata, the latter being rather long (about 16 by 3·5 μ) but not very characteristic. According to Stoll, there are two forms of the smooth, yellowish conidia, one being spherical (about 6.5 μ in diameter), the other pear-shaped (10 by 6 μ). Saccardo mentions spherical conidia, sometimes warty (5–7 μ). These statements, however, require correction, the conidia being

extremely variable in cultures on different substrata, both elongated, pear-shaped, ellipsoidal, spherical, smooth, prickly and warty forms being met with. The typical development on a good substratum (wort gelatin), however, furnishes ripe conidia that are decidedly warty globules with broad stems, the globules themselves soon falling asunder. At an earlier stage they are elongated, sometimes pointed, and also provided with a decided stem (*see also* Fig. 177). Mature specimens measure 6.8–9.2 by 5.7–6.8 μ. Consequently these conidia differ in a marked degree from those of all other species of *Penicillium*. No sclerotia ascospores have yet been observed. The species grows at only a moderate rate on the usual bacteriological substrata, and liquefies gelatin. The optimum temperature of growth is about 20°–23° C., and development is sluggish below 15° C. According to Stoll, a decided liberation of ammonia is produced on alkaline gelatin, but not on sugar or acid gelatin. Occasionally the spores are produced directly on the mycelium, without sterigmata or supports (*see* drawing). This observation needs reinvestigation, as indeed does the whole morphology of the species, the existing communications on the subject being scanty.

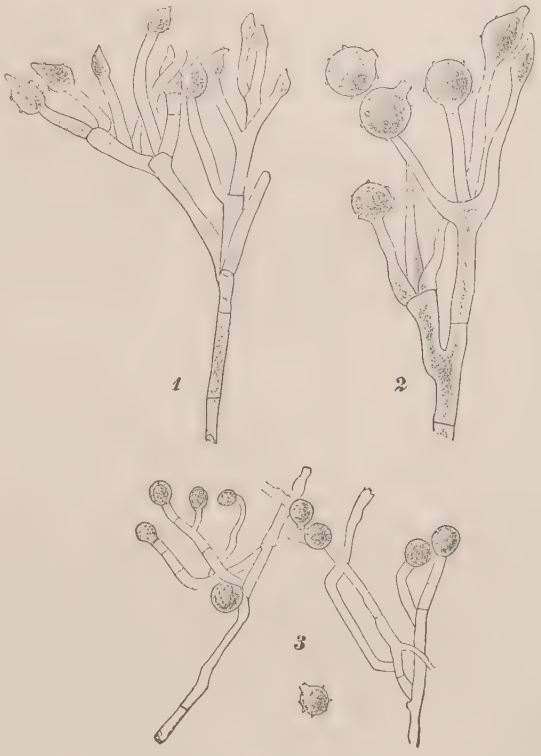


Fig. 182.—Penicillium brevicaule.

Formation of conidia on special conidiophores (1 and 2), as also directly on the mycelium (3), the former on wort gelatin, the latter on an agar-agar culture. The conidiophore 1 carries only young, elongated conidia in course of development, those on 2 being ripe and partly shed, together with three younger ones not yet septated (*see also* Fig. 177, 2). Approx. magn. of 1 and 2, 500 and 800 respectively; of 3, 400. (*Original.*)

The following species are less known, apparently scarcer and of no practical importance:

Penicillium purpurogenum, Stoll, was described by Stoll (I.), who obtained the species from Král, according to whom the original culture was isolated by Fleroff from impure Japanese koji. In respect of the conidiophores, colour of the vegetation, and the formation of pigment, it resembles *P. luteum*. The vegetation is dark green to dark greyish green; the conidiophores are delicate and branched in whorls, each twig being usually provided with four elongated, pointed sterigmata (7 by 2 μ). The conidia are ellipsoidal, very small (2.8 by 1.7 μ), and uniform in size and shape. The optimum temperature is about 30° C., growth ceasing below 15° C., though the fungus continues to develop at incubation temperature. It produces a yellowish red to purple-red pigment, but

only on substrata containing carbohydrates. The sterile mycelia are bright yellowish red.

Penicillium rubrum, Stoll, is a species of unknown origin, isolated by Grassberger, and described by Stoll (I.) in 1904. The hyphæ are coloured yellow to yellowish red by excreted granules; the conidial herbage dark green, and the conidiophores are delicate and often branched in whorls, the ends of the twigs carrying 4–5 long, pointed sterigmata (9.6 by 2 μ). The conidia are globular, very small (2.3 μ in diameter), and joined in readily detachable short chains. The optimum temperature is 30°–35° C. The species continues to grow at incubation temperature, but does not develop below 15° C. It produces a yellowish red to rusty brown pigment, but only on substrata containing carbohydrates, and the pigment differs from that of the preceding species. No fructification bodies have been discovered. Particulars of its behaviour in cultures, as compared with the two preceding species, are given by Stoll (I.).

Penicillium bicolor, Fries, is a species described by Oudemans (IV.) as having been isolated from soil; and, unless found to be identical with some other species, should probably be re-named. It forms greyish green coatings or cushions, with a sulphur-coloured rim (similar to *P. luteum*), but the conidia are spherical (2.3 μ in diameter). The conidiophores are divided up into two or three groups of fours, with cylindrical, pointed sterigmata and long conidial chains. It is probably difficult now to say what species was actually examined by Fries, though the yellow periphery of the cushions distinguishes it from "*P. glaucum*."

Penicillium claviforme, Bainier, was observed by that worker (I.) in 1905, on powdered oak bark at a drug store. The species is rendered so remarkable from the formation of club-shaped growths (1–2 cm. in height), which are white at first, but afterwards green, resembling those of *Isaria*, and also occurring extensively in pure cultures, that is not easily confounded with any other kind. The conidia measure 4.2 by 3.1 μ, and are of a pure colour. The species should probably be classed with *Isaria*.

Penicillium granulatum, Bainier, was observed on oak chips in the woods. It produces a yellow pigment. The conidia are globular to ellipsoidal, measuring 2.6 by 2.1 μ. This species, like the preceding one, was cultivated by Bainier (I.).

The following species need more accurate description, and are probably to some extent synonymous or doubtful.

Penicillium roseum, Link, forms reddish growths on vegetables. According to Oudemans (I.) the conidia measure 5–6 by 2–2.3 μ. The species is apparently rare, and also requires further investigation.

Penicillium radiatum, P. Lindner, differs from the other species by its tough-walled, dark-coloured conidiophores. The conidia are green and spherical. This species was found on cranberries by P. Lindner (XXXIII.), on which habitat it forms black, spherical sclerotia. Further investigation is needed for more complete particulars.

Oudemans (II.) has recently found (in 1902) several species in forest humus. These, however, can only be briefly mentioned, more complete investigation being necessary; from the description and drawings given, it is doubtful whether they really constitute new species.

Penicillium geophilum, Oudemans, produces conidiophores about 360 μ high and 6 μ thick, which are septated and provided with a whorl of bottle-shaped twigs (sterigmata), up to 30 μ in length, from which the conidia separate direct by abstriction. The conidia are spherical, green in colour, and 3–4 μ in diameter. The species, with its unbranched conidiophores, moreover, belongs to the genus *Citromyces*.

Penicillium humicola, Oudemans, is yellow-green. The conidiophores measure about 110–120 by 1–1.5 μ (probably a typographical error ?), and are branched in whorls. The conidia are 2 μ in diameter. Oudemans' diagrammatic drawing exhibits no special features.

Penicillium desciscens, Oudemans, is similar to the preceding species. The conidiophores show repeated branchings. The description gives the diameter of the conidia as 2–3 μ, but, according to the drawing, they are ellipsoidal (*P. luteum ?*).

Penicillium silvaticum, Oudemans, is brown. The conidiophores measure 210 by 2–3.5 μ, and are septated, with a whorl of bottle-shaped sterigmata, from which separate the pale brown, spherical conidia, 2–3 μ in diameter. The unbranched conidiophore excludes this species from the *Penicillium* group. It is apparently identical with *P. geophilum*, and should be classed with *Citromyces*.

Penicillium candidum, Link, forms white herbages, the conidiophores and conidia apparently coinciding with those of "*P. glaucum*." The spherical conidia measure 2–3 μ in diameter. The species grows on all kinds of vegetables. Accurate reports are lacking, but Morini (I.) speaks of sclerotia and asci, the latter being ovoid, 24–30 μ long, and containing eight smooth ovoid spores measuring 6.5–9 by 3.5–5 μ.

According to Roger (I.) a white *Penicillium*—termed *P. candidum*, but not fully described—plays a part in the ripening of Brie cheese, which it covers with a pale herbage. Epstein (I.), who also discovered this fungus, named it *P. album*, but its identity with the older *P. album*, Preuss (1851), has not been established; and it is also doubtful whether it coincides with the above-mentioned *P. candidum*, Link. More probably it is the same as *P. Camembert*, which is slightly green at first, afterwards turning greyish white.

Penicillium Duclauxii, Delacroix, forms herbages, which are white or sulphur-yellow at first, afterwards turning olive-green. The sterigmata are spindle-shaped, the conidia rounded-ellipsoidal, and measuring 3–4 μ in diameter. The species has been found on grapes that have lain in water. According to the description by Delacroix, the species is probably *P. luteum*.

Penicillium insigne (Winter), Schröter, forms white conidial herbages and elongated ellipsoidal conidia. The structure of the conidiophores closely resembles that of *P. luteum*. It was described by Winter (IV.) as *Eurotium insigne*, and, according to Schröter (I.), is identical with *Gliocladium penicilloides*, Corda. The pale yellowish brown, globular perithecia (0.25–1 mm. in diameter) have a smooth, thin, pseudo-parenchymatic skin. The asci are elongated ellipsoids (35–50–28–35 μ), and contain eight pale brownish yellow, spherical, prickly, tough-skinned spores, 15–20 μ in diameter. The species is found on the excrement of dogs and geese.

Penicillium aureum, Corda, forms yellow conidial herbages, turning to olive green, with very small, oval to spindle-shaped conidia (3–1.5 μ). The perithecia are thin-skinned, similar to those of the preceding species, but enveloped in a yellow covering of matted hyphæ. The spores (5 by 3 μ) are smooth, yellow, and ellipsoidal. Corda observed the species on decayed wood, and van Tieghem (III.) found it on the husks of *Bertholletia*. Further investigation is necessary, though in many respects it so closely resembles *P. luteum* that the two might be considered identical.

Penicillium Wortmanni, Klöcker, forms ascospores, which are stated by Klöcker (VI.) to be similar to those of *P. luteum* and *P. aureum*, though they are not smooth or provided with transverse ledges, but with stumpy warts, as in the case of *P. insigne*. How far the resemblance to the latter extends cannot be decided until a more complete description of the conidiophores is available.

The *Penicillium aromaticum*, observed by Johan-Olsen (III.) during the

ripening of Norwegian "gammelost," but not described, is probably nothing more than the *Penicillium* of Roquefort cheese. Particulars are also lacking of the *Penicillium* forms—which certainly included the Camembert *Penicillium*—observed by Costantin and Ray (I.) in Brie cheese. According to the results of investigations by de Seynes, the *P. cupricum* of Trabut (1895), is merely a form of the ordinary "*P. glaucum*," modified by the substratum (copper sulphate solution).

§ 288. The Genera Citromyces and Allescheria.

The genus *Citromyces*, Wehmer, comprises only a few forms, some of which are remarkable, physiologically, for their energetic power of acidification. It differs from *Penicillium* by the absence of branchings and by the swelling of the conidiophores (often into a globular form), and from *Aspergillus* by the slenderness of these organs and by the successive development of the sterigmata. The globule is spherical, club-shaped or insignificant. The conidiophores resemble hyphæ, are mostly aseptate, and, especially in aged specimens, provided with a colourless, thin-skinned, terminal, club-shaped to spherical globule. They are usually simple, slender, and project in large numbers from the mycelial filaments, the stalks being delicate and barely distinguishable from the vegetative hyphæ. The slender, tapering sterigmata are disposed in 5–10 whorls or tufts, pointing upward and inward, so that the head, deprived of conidia, resembles a calyx. The conidia are mostly spherical, very small (under 3 μ), green in the mass, and arranged in long chains. Ascospores are unknown.

Citromyces Pfefferianus, Wehmer, is undistinguishable from "*Penicillium glaucum*," even in colour, by the unassisted eye. It occurs as a tough, pure green mould, turning to greyish green, grey or brownish with age, on sour fruit, sugar solutions, sugar preserves, and lemon-juice; and in the open air as a fine green coating, occasionally on old mushrooms, &c. (*e.g.*, *Pholiota squarrosa*). The delicate, colourless conidiophores, measuring 3 μ in diameter (*see* Fig. 183), are scarcely 70 μ high, forming a dense herbage and carrying a globule, 4–8 μ across. On this the sterigmata, 9–14 μ long and about 3 μ thick, are arranged in a whorl or are irregularly distributed over the surface, a considerable part of which is usually left exposed. All the parts are thin-skinned and colourless, the only exception in this latter respect being the conidia, which are globular and 2.3–2.8 μ in diameter. Ascospores are unknown. According to Wehmer (XXXIV.), this fungus converts the sugar of the nutrient solution into free citric acid—in which connection *see* chap. lvii. § 291.

Citromyces glaber, Wehmer, agrees in its principal characteristics with the preceding species. The vegetations are closely interwoven and produce an abundance of conidia, which are of a rather darker green and almost smooth on the surface, and not bristly like the first species, the under side being dark, and often fissured. The conidiophores have globules up to 15 μ thick; the sterigmata and conidia are indistinguishable from those of the other species. The fungus stains boiled rice by means of a yellow pigment; and, according to Wehmer (XXXIV.), it also incites citric acid fermentation.

Probably several other allied species exist that are undistinguishable except from their appearance and behaviour in cultures; and the forms with unbranched conidiophores, referred to in the literature as *Penicillium* species, should also be included in this class. The same applies to the old but indefinite *P. radians*, observed by Bonorden on rotting leaves, and also to two of Oudemans species mentioned above (*P. geophilum* and *P. silvaticum*), as well, perhaps, as to the *P. radiatum* of P. Lindner (XXXIII.).

Mazé and Perrier (I.) recently established four species (*Citromyces citricus*,

C. tartaricus, *C. oxalicus* and *C. lacticus*), without, however, specifying their morphological characteristics; so they cannot be regarded as species in the sense of the naturalist. These two workers, instead of adopting the morphological basis of classification, apply the term *Citromyces* to all fungi producing citric acid. The practice of establishing genera according to physiological characteristics is specially indefensible in the case of forms that are morphologically well defined, and would also completely break up our system of natural history.

The genus *Allescheria*, Saccardo and Sydow, differs from *Penicillium* by the

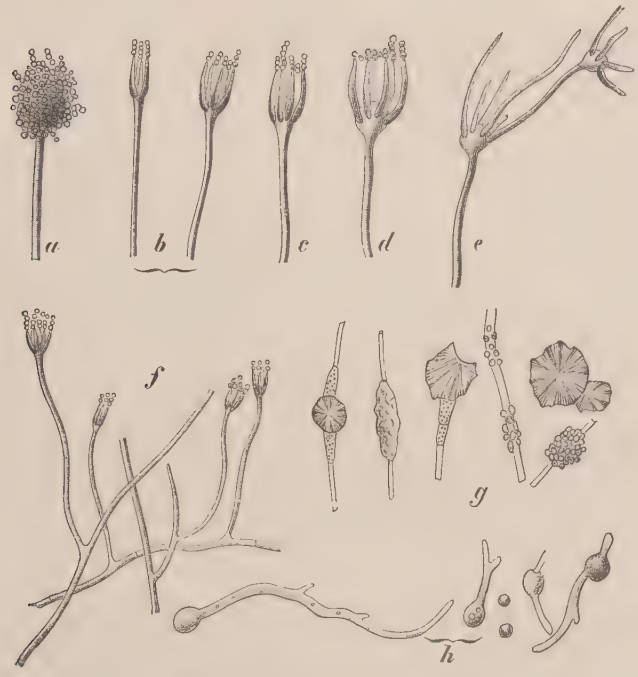


FIG. 183.—Citromyces Pfefferianus.

Conidiophores, at *b* and *d* after removal of the conidia, showing the variable globule with simple sterigmata; *e* is a malformation, a sterigma growing out into a new conidiophore. Conidiophores, slightly magnified at *f*. Hyphæ at *g*, from a growth in a calcareous nutrient solution, showing spherical, granular, or compact enveloping deposits of calcium citrate. Ripe and germinating conidia at *h*. Magn. of *a–e*, 400; of *f*, 240; of *g*, 400, of *h*, 600. (*After Wehmer.*)

sympodially branched conidiophores. Moreover, it is represented by only a single species. This was formerly described by COSTANTIN (IV.) as *Eurotiopsis Gayoni*, Cost., on the basis of a generic name already applied by Karsten to a genus of *Nectroidaceæ*, but should be named *Allescheria Gayoni* (Cost.), Sacc. and Syd. It also requires to be carefully compared with *Monascus purpureus*, Went. LINDAU (III.) proposed to call it *Eurotiella*; and ED. FISCHER (II.) has described it as *Allescheria Gayoni*, Saccardo and Sydow.

Allescheria Gayoni, Sacc. and Syd. (*Eurotiopsis Gayoni*, Cost.), is a species that has been more closely investigated by LABORDE (VI.) on account of its property of saccharifying starch. It produces a red pigment, and incites alcoholic fermentation, but though of chemico-physiological interest, has no practical importance, and will therefore be only briefly mentioned. It forms white or

reddish herbages, with sympodially branched conidiophores, developing long chains of ovoid conidia by abstriction. The conidia are relatively large, measuring 12 by 10 μ. The ascospores (resembling those of *A. glaucus* = "*Eurotium*") are spherical and small (50–80 μ in diameter), with rounded 8-spored asci, the spores measuring 6 by 4 μ. It forms purple-red patches on starch paste and other vegetable substrata In any event the conidium-producing form bears little resemblance to the previously mentioned *Aspergillaceæ*.

CHAPTER LVII.

CHEMICAL ACTIVITY OF THE ASPERGILLACEÆ.

By Prof. Dr. C. WEHMER.

§ 289.—General Review.

The present chapter deals specially with certain chemical effects of the *Aspergillaceæ*, many of the representatives of this family being worthy of note, having formed the subject of numerous investigations in this connection. This has already been discussed in various other parts of the present work, so that all we have to do now is to arrange the facts briefly, for the characterisation of the family in this respect as well.

The presence of enzymes, as the means of producing effects of decomposition, has been confirmed in all the species examined for this purpose. The number of enzymes identified seems to be still increasing, so that nearly all the enzymes known are found associated in *Aspergillaceæ*. Instances of the decomposition of carbohydrates (disaccharides and polysaccharides), glucosides, fats and proteids, by invertase, maltase, lactase, amylase, (diastase), inulase, cellulase (cytase), pectinase, melecitase, raffinase, emulsin, lipase, protease, &c., have been noticed; and mention has also been made of lab enzyme, amidase and tannase, as well as of oxidising and reducing enzymes. *Aspergillus niger* and the collective species "*Penicillium glaucum*," have particularly served as the subjects of experiment in these researches; but similar observations have also been recorded with regard to *A. oryzæ*, *A. Wentii*, *A. glaucus*, *Penic. luteum*, and isolated instances of other species of *Aspergillus* and *Penicillium*, as well as in the case of *Allescheria Gayoni* (*Eurotiopsis Gayoni*). Unfortunately the value of the results has been considerably impaired by the uncertainty regarding the identity of the so-called "*Penicillium glaucum*" examined by the different authors. Only in a very few instances have the enzymes in question been actually isolated, their presence having been, as a rule, deduced from the reaction with the culture liquid or with extracts from the triturated growths of mould.

In addition to enzyme action, true fermentative action in the stricter sense is found in only a few species; and in only one, namely *Allescheria Gayoni*, has any decided alcoholic fermentation been observed up to the present. On the other hand, oxidising fermentations have been noted in several species, namely: oxalic acid fermentation by *Asp. niger*, citric acid fermentation by *Citromyces Pfefferianus*, *Citr. glaber*, and *Penicillium luteum*. Whether these phenomena are separable from the living fungus and can be produced by the lifeless substance has not yet been investigated.

The dissociation of racemic compounds into their optically active components by micro-organisms has been already dealt with in vol. i. chap. xxii. The circumstance is only referred to now because nearly all the determinations were made by the help of *Aspergillaceæ*, more particularly *Asp. niger* and "*Penicillium glaucum*," though in many cases the purity and identity of the species may be doubted. Several experimenters have also worked with *Asp. flavescens* (probably *A. flavus*) and *A. griseus*, the latter name possibly masking the identity of some

better-known species (*e.g.*, *A. fumigatus*, Fres.). Unfortunately, descriptions of the fungi are lacking, so that the results are practically worthless. Attention has already been drawn to the circumstance that the *Penicillium glaucum* of the earlier workers was an imperfectly identified species, and can only be regarded as a collective term applied to green moulds of indefinite nature.

Our knowledge of the pigments produced by several of the species, and the conditions under which these pigments are developed, is still in an imperfect state. The same also applies to the poisons formed by the pathogenic species, though the less important decomposing action of several species on readily oxidisable substances (alcohols and organic acids) has been repeatedly examined. The chemical activity of our fungi is almost invariably connected with the presence of atmospheric oxygen, submerged vegetations being unable to bear the complete exclusion of oxygen for more than a short time, even when sugar is administered. Further particulars of the processes are given by Pfeffer (III.) and Duclaux (XXI.), in the lectures on plant physiology by Jost (I.), and in Czapek's (IV.) recently published work on the biochemistry of plants.

§ 290. Saccharification of Starch.

The diastatic property of the *Aspergillaceæ* is rightly placed in the foreground as the one of greatest practical importance. It has been utilised technically from the oldest times; and special historical interest attaches to the diastase of the Japanese *Aspergillus oryzæ*, this being the first enzyme from thread fungi to become better known, and forming the pioneer of the long series of fungus enzymes discovered during the last two decades of the nineteenth century. In 1860, Berthelot isolated yeast invertase, and the property of inverting saccharose, possessed by the extract from "mould fungi," was mentioned in 1864 by Béchamp. After Gayon's discovery of the inverting action of *Asp. niger* in 1878, the subsequent investigations of that fungus were not commenced until the eighties.

In 1876, Korschelt (II.), who was the first to publish a complete description of the method of saccharifying rice with *Asp. oryzæ*, practised in Japan, not only mentions that a diastase, capable of converting starch into dextrins and maltose, is secreted in the hyphæ of that fungus, but also tried to ascertain the optimum temperature (40°–50° C.) for the action of this enzyme, which he named eurotin (from *Eurotium oryzæ*, the earlier name of the fungus), and which greatly resembles malt diastase. The statements in the literature—*e.g.*, by Oppenheimer (III.)—ascribing the discovery of this diastase to later workers, consequently need correction. From the begining of the eighties it received attention at the hands of the majority of investigators, viz.: Atkinson in 1881, F. Cohn in 1883, Büsgen in 1885, and Kellner, Mori and Nagaoka in 1889. Then came Takamine's endeavours to utilise the properties of this *Aspergillus* beyond the confines of his native land, and the researches (extending up to the present time) into *Aspergillus* diastase (Taka-diastase) and its capacity, especially in comparison with enzymes of other origin. Reference to this matter has already been made in § 242, dealing with the technical application of the enzymes. In addition it may be stated that the extract from *Asp. oryzæ* or from koji contains not merely an amylase, but a mixture of various enzymes, whose divergent effects (decomposition of saccharose, maltose, &c.) cannot be ascribed to a single enzyme, since Atkinson demonstrated dextrose to be a saccharification product.

The saccharifying influence on starch, that Effront (X.) claims to be stimulated by a suitable mixture of different substances (phosphates, aluminium salts, asparagin, &c.), is adversely affected even by small quantities of alcohol or common salt, though additions of 20–30 per cent. are required to suppress it

entirely. According to Kellner, Mori and Nagaoka (I.), 2 per cent. of common salt will lower the effect of the mixed enzymes to 50.2–58.3 per cent. of its original value, 20 per cent. reducing it to less than 10 per cent; whilst 2 per cent. of alcohol will bring it down to 82 per cent., 10 per cent. of alcohol—according to Kozai (II.)—to 50 per cent., and 28 per cent. of this reagent to 1 per cent. Less than 1 per cent. of free acids (lactic acid, hydrochloric acid) also produced complete retardation. These factors play an important part in the technical utilisation of the fungus in the preparation of rice-wine, Soya and Miso.

Asp. oryzæ is by no means the only amylolytic species of this family, the same power being apparently shared by most of them, though in a less degree. So far as the species have been examined, starch paste (with the usual additional nutrient substances) forms a suitable substratum for all, and therefore the presence of the enzymes capable of acting on that medium is indicated. It is hardly necessary to enumerate the whole of these species. Experiments of this kind were commenced by Duclaux (XXI.) in 1883 with *Asp. niger*, and afterwards with *A. glaucus*, "*Penicillium glaucum*," and *Allescheria Gayoni* (*Eurotium Gayoni*), by Fernbach (III.), Bourquelot, Hebebrand (I.), Laborde (VI.), and Wehmer (V.). Observations on the saccharifying properties of extracts from the mould vegetations have recently been communicated by Schäffer (IV.), with regard to a number of species (*Aspergillus niger*, *A. Wentii*, *A. fumigatus*, *A. glaucus*, *A. oryzæ*, *Penicillium glaucum*, *P. luteum*, *P. italicum*, and *P. rubrum*).

Fernbach (IV.) and Bourquelot isolated from the cultures or growths of *Asp. niger* the amylase (diastase) previously mentioned by Duclaux (XXII.). The first-named worker also found that the preparation obtained by precipitation with alcohol has its activity seriously impaired by even small quantities of free organic or inorganic acids. This may also explain the circumstance, observed by Wehmer (V.) that the liquefaction of starch by this fungus (which produces free oxalic acid), is sometimes incomplete. Duclaux (XXII.) states that the fungus will also corrode and dissolve raw starch by means of a maltase differing from the ordinary kind, dextrose being formed. According to Laborde (VI.), the enzyme in question (amylomaltase), from *Asp. niger*, *Penicillium glaucum*, and *Allescheria Gayoni*, is able to transform starch directly into dextrin and dextrose, and not, as in the case of malt diastase, into maltose—which substance it is also able to hydrolyse. In this author's opinion (which, however, has not been left unchallenged), the amylomaltase secreted by these three fungi is not only different from the maltase of barley malt, but is also a different substance in each case, a conclusion formed on the basis of comparative behaviour under external influences, such as the action of acids and the optimum and maximum effect produced. This point, however, needs further investigation. Petit (IV.) states that both *Penicillium* and *Aspergillus* also convert into dextrose the dextrin $(C_6H_{10}O_5)_3$ formed during the saccharification of malt. Hebebrand (I.) has written on the diastase of *Penicillium;* and Gosio (VII.) on *Penicillium brevicaule*, which also saccharifies starch.

With regard to the conditions under which diastase is formed by *Asp. niger* and *Pen. glaucum*, reference may be made to pp. 46, 47, vol. ii. The continuous production of amylase in cultures of *Asp. niger* on sugar solution was assumed in 1889 by Duclaux, but no proof was advanced; so that the work of this experimenter, fruitful as it was, affords no experimental proof of the various new statements.

§ 291. Acid Fermentations.

In contrast with the various enzyme actions of the *Aspergillaceæ*, the production of free organic acids—the sole process to which we apply the term "acid

fermentation"—is a rare occurrence; and up to the present this is the only family of *Eumycetes* in which this process is carried on in the same way as by the bacteria. Just as in the latter case the chief products of this fermentation are acetic acid, butyric acid, and lactic acid, so with the *Eumycetes* the products are oxalic acid and citric acid. In the case of phanerogams, similar processes furnish preferably citric acid, tartaric acid or malic acid. The accumulation of such acids in any appreciable quantity, whether in the vacuoles of higher plants or in the nutrient solution of micro-organisms, is invariably a physiological peculiarity confined to certain species or families (*Aurantiaceæ*, *Crassulaceæ*, *Vitaceæ*, &c.), and one that is difficult to analyse closely. In the majority of cases the free organic acid is merely an intermediate product, which is afterwards decomposed by complete oxidation; and the question whether the formative stage has been traversed too rapidly or the decomposition stage too sluggishly must remain open for the present. Of course, the presence of salts of organic acids, which are commonly met with, does not necessarily imply acid fermentation, since the occurrence of these salts does not argue the pre-existence of free acids, but is more frequently the result of the availability of bases during metabolism.

At present we are probably only on the threshold of knowledge with regard to acid fermentation, and continued systematic investigation may reveal both additional fungi and acids concerned in the process. Even now a few reports—still, however, incomplete—are available on the point. Thus, it is known that *Asp. oryzæ* will acidify saccharine nutrient media, though the nature of the acid has not been determined. Graf (I.) found the acidity of a 28-days-old culture on wort to be equivalent to 40 c.c. of decinormal baryta per 20 c.c. of culture liquid, as compared with an acidity of only 2.45 c.c. in the case of "*Penicillium glaucum*" and of 56.6 c.c. with *Asp. niger*, for the same volume of liquid. The fact discovered by Lind (I.), that *Asp. niger* and "*Penic. glaucum*" will corrode thin plates of lime (*see* p. 44, vol. ii.), is apparently—as in the case of algæ, rich in calcium oxalate—at least partly due to such acidity.

The statement by Sanguineti (I.) that formic acid and acetic acid are present in cultures of *Asp. oryzæ*, lacks probability, and should be confirmed by means of pure cultures; and the same applies to Heinz's (II.) assumption that acetic acid is produced by *Asp. niger*. Moreover, the statement that a so-called *Lactomyces* fungus will ferment sugar solutions to lactic acid must be regarded as lacking both proof and probability, although the German authorities granted a patent (No. 118,063, of Feb. 26, 1901) for it, especially as no thread fungi have yet been found to produce that acid. This opinion is endorsed by Czapek (IV.). The souring of culture liquids by certain *Mucorineæ* has already been dealt with in the present volume (p. 53). The *Aspergillaceæ* recognised as producers of free acids include primarily *Asp. niger*, *Penicillium luteum*, and two species of *Citromyces*, the first of these furnishing oxalic acid and the last three citric acid.

Decided oxalic-acid fermentation has, so far, been observed solely in the case of *Aspergillus niger*, merely indications being found with *Asp. glaucus*, *Penicillium glaucum*, and also with certain non-*Aspergillaceæ* (*Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Rhizopus nigricans*), the slight traces of an excess of free acid being only prevented from further decomposition by immediate neutralisation. In other groups of the vegetable kingdom, however, free oxalic acid is formed during metabolism, and this acid may remain free (*Rheum* and also *Oxalis* species), though it is usually thrown down at once by the calcium carbonate supplied by the soil-water (Cacti, the buds and bark of various shrubs, algæ). The application of the term "fermentation" to the process in the case of fungi does not affect its similarity of character in all these cases, though, of course, it must not be classed indiscriminately with the formation of oxalates.

The mere occurrence of calcium oxalate crystals in fungi has long been

known, and frequently observed in cultures of *Asp. niger*, this being also reported by Schroter (II.). Their origin in gelatin cultures of *Penicillium glaucum* was mentioned by A. Hansen (I.) in 1889, and in sclerotia of the same fungus by Brefeld in 1874. A. de Bary (II.) referred to the formation of soluble oxalates by *Sclerotinia sclerotiorum;* and Duclaux (XXII.), in 1889 mentioned casually (and without any experimental proof) the formation of oxalic acid or oxalates in cultures of *Asp. niger* on different substrata. Zopf (XIV.) in 1889 found oxalate crystals in cultures of a species of yeast, and also in cultures of various acetic bacteria in peptonised sugar solutions with added gelatin; and thoroughgoing observations on this point were published by Banning (I.). The proof that oxalic acid in the free state is produced by fungi, especially *Asp. niger*, solely in presence of carbohydrates or chemically allied substances, was afforded by Wehmer (V.) in 1892; and at the same time an attempt was made to bring the previously known facts to a focus and refute the budding hypotheses on the relation between the occurrence of oxalic acid and the formation of protein. These discoveries led to a series of definite determinations on the oxalic fermentation of this fungus, the result being to rank the process with other fermentations. According to Wehmer (XXVI., XXVI*a*, and V.) the process goes on in the following manner:

As soon as the vegetation has developed from the sown spores, the nutrient solution of the pure culture of *Aspergillus* at room temperature begins to turn red Congo paper blue, and to liberate gas in presence of calcium carbonate—both certain reactions for the presence of free acid. The acidity gradually increases to a maximum, declining once more during the next few weeks, to gradually fall to zero when the experiment is prolonged, the reaction being even alkaline finally. The capacity of this fungus for destroying free acid can be demonstrated by placing the mature vegetable growths on dilute solutions of oxalic acid (containing 0.5 per cent. of crystalline acid). The limit of the accumulation of acid averages about 0.2 per cent. of the volume of the liquid. The amount of sugar is immaterial, but the general conditions of the environment are important.

The acidification is primarily dependent on the organic nutriment presented, sugars, or chemically allied substances being essential, whereas no free acid is produced when salts of organic acids, amides or peptone are used, though an abundance of oxalates is formed. A decisive influence is also exerted by the inorganic bodies present, especially the source of nitrogen for the growing fungus, the liberation of acid being absent when ammonium chloride or sulphate is substituted for potassium, calcium, or ammonium nitrate (even in presence of sugar); and, in fact, these additions will prevent the formation of acid in cultures that would otherwise acidify at once. Temperature also plays an important *rôle* from the outset and has a determining effect on success, lower temperatures favouring the accumulation of acid, whilst high temperatures have an adverse effect, so that at the optimum temperature for the growth of the fungus (about 37° C.), acidification ceases to occur, the highest production (up to about 1 per cent.) being attained at a few degrees above the minimum growth temperature (about 7° C.). The fungus being actually capable of far more readily decomposing free oxalic acid at higher temperatures (and even when 0.4 per cent. is present), the accumulation at lower temperatures is therefore solely the result of retarded oxidation, that is to say, enfeebled oxidising action. The whole shows clearly that, contrary to preconceived ideas, a relative scarcity of oxygen is not the cause of the production of oxalic acid, since all the growths in these experiments had an equal supply of oxygen, and the acid must therefore be regarded as the product of incomplete oxidation only in the sense that this oxidation has been prevented by some adverse influence or other.

A remarkable influence is exercised on the process by the addition of salts

able to combine with the acid. In this case the accumulation of the combined acid is progressive, and finally attains extraordinary dimensions. The resulting calcium oxalate may amount to more than 100 per cent. of the sugar originally present, so that 15 grms. of sugar furnish about 10 grms. of (anhydrous) oxalic acid (corresponding to about 7 grms. of sugar), without the crop of the fungus being affected. In this way the fungus produced the following quantities of calcium oxalate from 1.5 grms. of grape sugar in presence of added chalk, at 15°–20° C.:

After 11 days	0.282 grm.	After 72 days	1.340 grms.
„ 16 „	0.570 „	„ 100 „	1.642 „
„ 27 „	0.650 „	„ 120 „	1.615 „
„ 46 „	1.122 „	„ 247 „	1.730 „

In the absence of added chalk, the amounts precipitated from the same nutrient solution were only:

After 9 days	0.005 grm.	After 66 days	0.298 grm.
„ 16 „	0.070 „	„ 78 „	0.130 „
„ 23 „	0.170 „	„ 97 „	0.103 „
„ 46 „	0.255 „	„ 120 „	0.018 „
„ 54 „	0.248 „	„ 175 „	0.014 „

At higher temperatures (34°–35° C.), under otherwise equal conditions, only traces of oxalate were formed, viz.:

After 4 days	0.000 grm.	After 32 days	trace
„ 8 „	0.008 „	„ 42 „	„
„ 15 „	0.028 „	„ 68 „	0.068 grm.
„ 18 „	0.000 „		

On the other hand, at 7°–9° C., without chalk, 0.624–0.820 grm. was found after about seven months; and with chalk, even at a temperature of 34°–35° C., there was obtained from the same amount of sugar (1.5 grms.), 1.133 grms. of calcium oxalate at the end of forty-six days, and 1.340 grms. after seventy-two days.

In order to withdraw the acid from the further action of this physiologically interesting fungus by fixation, it is not even necessary to convert it into an insoluble salt, the same effect being produced by the aid of soluble salts, such as alkali phosphates of alkaline reaction, and even neutral alkali phosphates, the latter being transformed into acid oxalates. Apparently the appearance of alkali acid phosphates in phanerogams (*Oxalis*, &c.) is based on this circumstance. Conversely, it is interesting to find that *Penicillium glaucum* decomposes both free acids and alkali oxalates much more readily than *Aspergillus* does, and, therefore, if for no other reason, cannot be a generator of acidity to any extent.

Although, in general, experiments of this kind with *Asp. niger* proceed with the certainty of a chemical test, variations are not unknown in individual cases. The isolated instances in which Wehmer (XVII.) and Emmerling (VI.) found no acidification must probably be allocated to this category. Other factors may, perhaps, have contributed, since, according to a previous discovery by Wehmer (XXVIII.), the addition of even a trace of iron salts to cultures grown in the light can favour the redecomposition of the acid. References to the formation of oxalic acid by *Asp. niger* are also found in certain recent investigations, such as in those of Emmerling (VI.) and Heinze (II.). We have naturally excluded here the cases in which the production of oxalic acid is regulated by the liberation of bases during metabolism (*e.g.*, the salts of other organic acids, amides, peptones, &c., are consumed). In such cases oxalates are produced instead of any surplus of free acid, the process here—which is independent of temperature

—being of a different character, and in the absence of the liberated bases no accumulation of acid would take place. To this class of process relate several earlier and later investigations mentioned in the literature, where the workers, *e.g.*, Zopf (XIV.), Banning (I.), &c., employed additions of amides, peptones, meat extract or gelatin, instead of merely sugar solutions and mineral salts. In presence of actively oxidising organisms, the chemical nature of these substances necessarily entails the formation of oxalates or carbonates. Up to the present, no competitor exhibiting the same decided peculiarity as *Asp. niger* has been found among the thread fungi, yeasts or bacteria.

A work on the production of oxalic acid by *Asp. niger*, published in 1905 by Charpentier (I.), who was unfortunately not acquainted with the existing literature of the subject, merely repeats what was already well known; and his remarkable conclusion that the production of acid is a result of the exhaustion of the nutrient medium shows such an inaccurate conception of the true state of affairs as to require no serious refutation. According to Heinze (II.), acetic acid is formed along with the oxalic acid—a statement requiring further confirmation, at least so far as pure cultures are concerned. In view of the ease with which acetic acid is decomposed by the fungus in question, as reported by Pfeffer (VI.) and Duclaux (XXII.), this formation is not very probable, nor has it been properly demonstrated. Moreover, the circumstance that in Heinze's three experiments with a two-fold and three-fold quantity of nutrient solution, the amount of oxalic acid was correspondingly greater than with 200 c.c., is—as emphasised by Wehmer—the natural result of the regulation of the production of acid, and not due to the lower content of nitrogen. That Heinze observed the formation of potassium nitrate by this fungus from peptone, gelatin, &c., is incredible from the sum of his reports. The brief statements of Kostytschew (I.) regarding the production of acid by the intramolecular respiration of *Aspergillus* are of too general a character to allow definite conclusions to be drawn from them.

Citric acid fermentation ranks along with that in which oxalic acid is produced, and relates solely to the formation of free citric acid, but not to the production of citrates so generally observed among the phanerogams and fungi. A parallel to the exciters of this fermentation is found among the phanerogams, in the *Citrus* species, just as *Aspergillus niger* is physiologically allied to the *Rumex* and *Rheum* species. We shall now deal concisely with the chemistry conditions and course of the process, as observed by Wehmer (XXVIII.), more particularly in the case of *Citromyces Pfefferianus* and *Citr. glaber*. According to the same worker (XXIX.), both *Penicillium luteum* and *Mucor piriformis* are feeble acid-formers.

Being an oxidation fermentation, the process is dependent on the presence of an abundance of oxygen, in the same degree as the acetic and oxalic acid fermentations. When air is excluded, neither *Aspergillus* nor *Citromyces* spores will develop at all, and even the mature growths only survive a short time under these conditions. Although citric acid must also be regarded as the product of an incomplete oxidation, its formation is not the result of an insufficient supply of oxygen, but the consequence of a decomposition arrested through other causes. True, the temperature does not seem to be such a decisive factor as in oxalic acid fermentation, and further determinations are necessary in order to elucidate the dependence of the process on external conditions. In this case also the chemical character of the organic nutriment is an essential factor, carbohydrates or allied substances alone, and not peptones, amides, salts of organic acids, &c., enabling the production of free acid to take place.

In its incipient stage, the acidification, which takes place without any visible liberation of gas, can be detected by the blue reaction with Congo paper; and an

addition of chalk produces brisk effervescence. The acidity gradually increases, the limit being reached at about 8 per cent., without any apparent influence on the development of the fungus. The latter then begins to redecompose the accumulated acid, and the acidity decreases, no trace of free acid being perceptible at the end of a few weeks longer; hence it is undoubtedly merely an intermediate product that has momentarily escaped further decomposition. In this case, also, the anticipated effect of fixing the acid in the form of salts is realised, neutralisation preserving the acid from redecomposition; at the same time the formation of acid is accelerated and the total amount is considerably increased. Consequently, whilst the acidity increases but slowly in the absence of chalk, the addition of this substance to the acidifying culture results in a continuous liberation of gas, much more apparent than in the case of oxalic acid fermentation, and followed in a short time by an extensive deposition of calcium citrate.

The citrate is separated from the unaltered calcium carbonate by dissolving it in hydrochloric acid, neutralisation with ammonia, and boiling—which precipitates the citrate—the mass being dried at 110° C. and weighed, so that the quantitative yield can be approximately determined. The average weight is from one-third to one-half the quantity of sugar, in the form of the crystallised acid (with 1 molecule of water), this being recovered in the usual manner by freeing it from the lime salt with sulphuric acid, filtering from the resulting gypsum, and concentrating to the point of crystallisation. Hence, in well-conducted experiments, nearly half the sugar is converted into citric acid, without any appreciable hindrance to the development of the fungus. As we have seen, *Asp. niger* is also able to transform about one-half the sugar (dextrose) into oxalic acid, the resulting weight of acid being more than three-quarters that of the original sugar.

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} + O_3 = \underset{\text{Citric acid.}}{C_6H_8O_7} + 2H_2O$$
180 192 (+ 1 molecule H_2O = 210)

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} + O_9 = 3\underset{\text{Oxalic acid.}}{C_2H_2O_4} + 3H_2O$$
180 270 (+ 2 molecules H_2O = 306)

In both cases about one-half the material is consumed in satisfying the needs of the fungus; but possibly an alteration in the conditions of experiment might increase the proportion of the product. The resulting calcium citrate remains at first dissolved in the culture liquid, and it is only as the concentration increases that it separates out largely in the form of a bulky crust, consisting of coherent acicular or granular concretions, at the bottom of the vessel. When precipitated in the above manner from hot solution, it has the composition $(C_6H_5O_7)_2Ca_3 + 4H_2O$, containing therefore about three-quarters of its weight of crystallised acid $(C_6H_8O_7 + H_2O)$, which is recovered in a pure state, free of impurities.

With reference to the technical importance of a process of this kind for manufacturing citric acid, it may be mentioned that the market price of the acid is about six guineas per cwt., whilst that of the raw material is only about one-tenth that figure. Nevertheless, there are certain difficulties, not easily overcome in practice, with respect to the nature of the apparatus required for manufacturing large quantities of acid, as well as in connection with the risk of infection and the fluctuating character of the fermentative power. The "Fabriques de Produits Chimiques" at Thann and Mülhausen, under the management of Scheurer-Kestner, have been occupied with this question for a long time.

The chemistry of the process also merits a brief description. The conversion of sugar (dextrose) into citric acid is a matter not merely of oxidation, but of the simultaneous splitting up of the normal carbon chain of the sugar molecule. In accordance with the formula of the acid, the one carbon atom is transferred to a side chain:

COOH	COH
CH_2	CH.OH
COH.COOH	CH.OH
CH_2	CH.OH
COOH	CH.OH
Citric acid.	CH_2OH
	Dextrose.

Hence the process appears more complicated than other fermentations; and, contrary to statements in the literature, no one has yet succeeded in obtaining the acid by the simple oxidation of sugar.

There is little to be gained by going into the question of the biological importance of such acid fermentations, since it affords no explanation. Although, in the case of oxalic acid fermentation, it might be that the accumulating acid is injurious to competitors for the available food, this can hardly apply to citric acid fermentation. In both cases the acid is lacking at the time when it would be most effective, namely, at the commencement of vegetation; and where it is afterwards present in abundance, it no longer possesses any value in this respect, the fungus having already fully occupied the substratum. Moreover, the accumulation injures the fungus, and finally, liquids containing citric acid also permit the development of certain other fungi. A far more important problem is whether the organism is not itself injured by the waste of substance entailed by the accumulation of acid, as is certainly the case, for instance, in alcoholic fermentation. This does not appear to be so with oxalic acid fermentation, and, according to Wehmer (XXVIII.), it is not appreciable in the case of citric fermentation, despite the higher physiological value of this acid, the only way in which it can be estimated being by careful quantitative determinations. In general, however,—from the standpoint of practicability—it may be said that in the interests of the organism fermentations of this kind are better dispensed with, since they imply a more or less uneconomical utilisation of the substratum.

Mazé and Perrier (I.) have latterly occupied themselves with the formation of citric acid. They observed this to occur from alcohol and glycerine, and ascribe its origin to an incipient scarcity of nitrogen in the culture, though it is independent of the presence or absence of oxygen. The acid is said to result from a process of disassimilation when the substratum has been exhausted of assimilable nitrogen; and its formation is preceded by a decomposition of the sugar into alcohol and carbon dioxide. It would probably be easy to refute these statements by experiment. Mazé and Perrier seem to be acquainted with only a preliminary communication by Wehmer (XXX.), and not with his more exhaustive work (XXVIII.).

§ 292.—The Fission of Disaccharides and Trisaccharides, Glucosides and Polysaccharides (Starch Excepted).

The inverting enzyme of the *Aspergillaceæ* was discovered a few years anterior to their diastasic enzyme; and subsequently various other enzymes were recognised. Up to the present, these have chiefly been detected in *Asp. niger* and *Pen. glaucum*, the latter being invariably a collective term for a number of very similar species not more closely identified.

Asp. niger splits up all the sugars here in question (except milk sugar, which still remains doubtful) before consuming them, and therefore secretes invertase (sucrase), maltase, trehalase, melecitase (?) and raffinase. This has been demonstrated by a series of observations, dating from the year 1878, by Gayon (V.), Duclaux (XXII.), Fernbach (V.), Bourquelot, Hérissey, and Gillot (III.). Bourquelot especially—both alone and in collaboration with Hérissey, and Graziani—has repeatedly investigated these enzymes, and enriched our knowledge of them. The first report of the inversion of saccharose by the fungus dates from 1878, namely, by Gayon (V.); and Duclaux has repeatedly treated the question since 1883. According to Fernbach, who detected the enzyme in 1890, and also examined the deterrent influence of light on its action, the formation of invertase is *not* dependent on the presence of saccharose in the culture liquid. In 1893 it was studied by Bourquelot; and in 1896 this worker, in association with Hérissey (I.), demonstrated the enzymatic splitting up of the trisaccharide melecitose into dextrose and turanose—the latter a disaccharide analogous to maltose, and one that cannot be further modified by the fungus, though it can be by the action of acids. The same worker obtained, in 1893 (also by alcoholic precipitation), an enzyme (trehalase) capable of hydrolysing trehalose into two molecules of dextrose, and differing from invertase and maltase though it may be identical with the amylase of E. Fischer (III.), which acts in the same way. Bourquelot finally demonstrated the enzymatic fission of maltose and raffinose (VII.)—the latter by means of a separate enzyme—and also (VI.) that of the trisaccharide gentianose into dextrose (2 molecules) and lævulose, subsequent to the intermediate formation of gentiobiose (capable of subdivision into 2 molecules of dextrose) and lævulose (invertase and emulsin), in part of which researches he collaborated with Hérissey (III.). With regard to raffinase, opinions are still divided, some considering its action limited to the splitting up of melibiose (Bau's "melibiase") into galactose and dextrose, and that the conversion of melitriose into melibiose and dextrose is accomplished by invertase. According to E. Fischer, it is very similar to maltase; with which melecitase is also probably identical. Furthermore, according to Bourquelot (V.), gentiobiose and turanose are also capable of being split up by specific enzymes. The action of maltase was also investigated by Hérissey (I.), and that of raffinase by Gillot (III.), both working with the same fungus. Consequently, a considerable literature exists on the sugar-decomposing enzymes of *Asp. niger* alone.

"*Penicillium glaucum*" behaves in a very similar way, this fungus having already been found to contain invertase (Duclaux, 1883), maltase and trehalase (by Bourquelot (VII.) and others since 1880), and raffinase (by Gillot (III.) in 1900). The optimum temperature (45° C.) of *Penicillium* maltase is about 30° lower than that of *Aspergillus* (Bourquelot). The observation made in 1864 by Béchamp (XII.), on the inversion of saccharose by the filtrate from crushed mould fungi, probably relates to "*P. glaucum*."

Bourquelot and Graziani (I.) failed to discover any invertase in the culture liquid in the case of the saccharose-inverting *Penicillium Duclauxii* (which is probably identical with *P. luteum*), the enzyme being, perhaps, retained by the mycelium.

A. oryzæ has long been known for its inverting power (Atkinson, 1881, Kellner, Mori and Nagaoka, 1889), by virtue of which it effects the enzymatic fission of maltose, though not lactose. According to Kozai (I.) it can also degrade raffinose (melitriose). Our present ideas on the subject no longer allow us to ascribe these effects to a single enzyme (the eurotin and invertase of older workers); and indeed Kellner doubted the uniform nature of "invertase." Lactic acid in small quantities (0.05 per cent.) acts as a stimulant in respect of the fission of saccharose, but even as little as 0.1 per cent. retards the action,

and 0.6–0.7 per cent. restricts it entirely; indeed the action sinks to about one-fifth with 0.5 per cent.—compare KELLNER, MORI and NAGAOKA (I.). The action of alcohol and common salt is probably about the same as in the case of amylase.

A special position is occupied by *Allescheria Gayoni* (= *Eurotiopsis G.*), inasmuch as it contains lactase, but not invertase. In addition, maltase and trehalase were detected in this fungus by LABORDE (VI.) in 1897. In the fermentation of solutions of invert sugar, the lævulose was attacked at an appreciably more rapid rate than dextrose. Up to the present this is the only member of this family of fungi that has been observed to split up lactose enzymatically, previous to consuming it. The question whether the same is done by *A. niger* and *P. glaucum* was discussed by DUCLAUX (XXII.), as long ago as 1889, but was left unsettled. More recent experiments by SCHÄFFER (IV.) also led to no definite result, although this worker thought he observed a slight action on lactose in the case of *A. niger*, *A. oryzæ* and *P. glaucum*. He also found that saccharose solution is inverted by the extracts furnished by vegetations of all the *Aspergillaceæ* examined (*A. Wentii*, *A. fumigatus*, *A. glaucus*, *A. oryzæ*, *A. niger*) and *Penicillium* (*P. luteum*, *P. rubrum*, *P. italicum*, *P. glaucum*), whilst lactose exhibits an incomparably greater resistance. He likewise states that maltose is converted into glucose by extracts of the fungi in question.

A good deal of information is also available on the fission of the glucosides, the formation of emulsin being mentioned in the case of *Asp. niger*, *A. oryzæ*, *A. fumigatus*, *A. Wentii*, *Penic. luteum*, *P. rubrum*, *P. italicum*, *P. glaucum*, and *Allescheria Gayoni*. This enzyme was also isolated from *Asp. niger* in 1893 by BOURQUELOT (III.), who—partly in collaboration with HÉRISSEY (II.)—studied its behaviour toward several glucosides: amygdalin, salicin, coniferin, helicin, populin, arbutin and æsculin; all of which were split up by the extract from the vegetative fungus, though negative results were obtained in the case of digitalin, solanin, hesperidin, convallamarin, jalapin, &c. The precipitate thrown down by alcohol from the solution concentrated *in vacuo* has the same effect. HÉRISSEY (II.), made a more exact comparison with almond emulsin, from which it differs in several respects, populin and phloridzin, for instance, being split up (into benzoyl, saligenin and phloretin respectively) by the *Aspergillus* emulsin alone. It, however, could not be separated from the other enzymes of this fungus. According to GÉRARD (VI.), *Penic. glaucum* also secretes an enzyme that can be isolated by lixiviating the fungus, acts like emulsin and splits up amygdalin and salicin. *Asp. glaucus* agrees with the two fungi just named in respect of its behaviour to solutions of glucosides. All three of the fungi were tested, in the form of living vegetations, by PURIEWITSCH (V. and VI.). On a solution of helicin the fungus died off under the influence of the resulting salicylic aldehyde, salicin was decomposed with formation of saligenin, the dextrose being consumed by the fungus at once; and similar results were obtained with arbutin, coniferin, æsculin, hesperidin and phloridzin. The fission of amygdalin into dextrose, benzaldehyde and hydrocyanic acid was observed only in the case of extracts, or after etherising the fungus, the living vegetations producing neither benzaldehyde nor hydrocyanic acid, so that in this case the fission seems to proceed in a different manner. The author believed that decomposition into sugar and amygdalic acid was effected by an enzyme allied to invertase; but this is certainly incorrect (*see* below). The secretion of enzyme was suppressed by the addition of larger quantities of sugar, which therefore prevents the fission of the glucosides in a manner analogous to the action of diastase on starch. J. BEHRENS (IX.) found emulsin in *Penic. luteum*; and this fungus also splits up quercitrin. According to LABORDE (VI.), the living vegetations of *Allescheria Gayoni* (*Eurotiopsis G.*) will split up the glucosides

(amygdalin, salicin and coniferin), with formation of sugar. Results differing to some extent from those of Puriewitsch were obtained by Brunstein (I.) in 1901, who tested *Asp. niger*, *A. oryzæ*, *A. Wentii*, *A. glaucus* and *Penic. glaucum* in presence of helicin, salicin, arbutin, amygdalin, coniferin, myrosin, saponin and glycyrrhicin, all of which were split up by the living vegetations, except myrosin, which gave doubtful results. *Asp. glaucus* and *Asp. Wentii* split up helicin, without formation of salicylic aldehyde, salicylic acid being produced; *Asp. niger*, *A. oryzæ*, and *P. glaucum* furnished salicylic aldehyde, this being oxidised to salicylic acid by *Asp. oryzæ*, &c. The latter product was in turn consumed by several of the fungi, especially by *Asp. Wentii*. On the other hand, the hydroquinone formed from arbutin had a poisonous effect. Amygdalin was split up by all the species into sugar and cyanhydrin, which underwent secondary oxidation to mandelic acid, with liberation of ammonia. The fission of amygdalin and helicin by the living fungus, especially by extracts from the vegetations, was also demonstrated in the same year (1901) by Schäffer (IV.), in respect of a larger number of species, *Asp. fumigatus*, *Penic. luteum*, *P. rubrum* and *P. italicum* acting in the same way, in addition to *Asp. niger*, *A. Wentii*, *A. glaucus*, *A. oryzæ*, and *P. glaucum*. In this case, also, potassium myronate was not attacked. The actual organism with which Hérissey (III.) obtained similar effects, namely, the so-called *Asp. fuscus*, Bonorden, is uncertain, Bonorden's description being insufficient for its identification.

The fission of the polysaccharides has also been investigated. The occurrence of an enzyme splitting up inulin was shown, in the case of *Asp. niger* and *Penic. glaucum*, by Bourquelot (III.) in 1893; but, according to Kellner, Mori and Nagaoka (I.), it is lacking in *Asp. oryzæ*, and, according to Laborde (VI.), in *Allescheria*, though the latter forms reducing sugars from gum-arabic. A closer investigation of this enzyme has recently been undertaken by Dean (I.), in the case of *Penic. glaucum* and *Asp. niger*. He finds that it does not issue from the hyphæ spontaneously, so that it belongs to the endo-enzymes. It is injuriously affected by acids and alkalis, even in small quantities; and its optimum temperature of action is given as 55° C. Schäffer (IV.) states that inulase is also secreted by *Asp. oryzæ*, *A. Wentii*, *A. fumigatus*, *A. glaucus*, *A. niger*, *Penic. luteum*, *P. rubrum*, *P. glaucum* and *P. italicum*. On the other hand, the enzymatic solution of "true" cellulose seems to be a matter of rare occurrence with all the members of this family; for, though it is true that Miyoshi (III.) observed bursting of the cell walls on the hyphæ of *Penicillium* being stimulated chemotactically, the same result occurs when mechanical pressure is applied (*see* p. 45, vol. ii.). J. Behrens (IX.) also confirmed the incapacity of *Penic. glaucum* and *P. luteum* to dissolve cellulose, though both were able to dissolve the substance of the middle lamellæ, and therefore—like *Asp. niger*—secrete pectinase. Two species, *Asp. oryzæ* and *Asp. Wentii* are reported as able to grow through the substance of soft-boiled rice and Soja beans; and according to Prinsen-Geerligs (I.), *Asp. Wentii* penetrates and dissolves the cell walls, setting the contents free. In this case, however, the material is not true cellulose, and consequently the nature of the enzyme has still to be determined. It has also been stated by Newcombe (I.), as well as by Okamura and Takakusu (I.), that *Asp. oryzæ*, secretes cytase (cellulase); but as the walls of the barley endospore, which were treated (by Newcombe at least) with the enzyme mixture from *Asp. oryzæ* (the so-called "Takadiastase") consist merely of a hemicellulose (Reinitzer) attackable even by malt amylase, this result is not decisive. In this case the walls were dissolved even before the starch (in twenty-four hours as compared with about eight to twelve days). Oppenheimer (III.) regards this case as one of cellulose solution, and (as reported by Miyoshi) has obtained the same effect with *Penic. glaucum*; but Miyoshi found precisely the opposite. Van Iterson

seems to have observed a very feeble action effected by *Asp. niger* on blotting-paper.

The fermentation of tannin, which was also considered to be a glucoside, was reported by VAN TIEGHEM (XIII.), in 1867, to be effected by two mould fungi, *Asp. niger* and *Penic. glaucum*. This worker considered that the fission of tannin into gallic acid and glucose was a "true fermentation phenomenon," *i.e.*, a manifestation of vital activity, and not the effect of a substance secreted by the mycelium of the fungus. FERNBACH (III.) and POTTEVIN (II.) afterwards demonstrated contemporaneously that this view is incorrect, and that *Asp. niger* secretes an enzyme (tannase) that is precipitable by alcohol, and is able of itself, in a sterilised solution, to split up tannin (digallic acid) into gallic acid, a yield of 98.7 per cent. of this acid being obtained from pure tannin. In cultures the sparingly soluble gallic acid separates in fine crystals from the tannin solution. This process has been patented (Ger. Pat. 13,187, of 1901) for the production of gallic acid on a commercial scale. The dextrose (12–15 per cent.) observed by van Tieghem as accompanying gallic acid in the product from commercial tannin is not a fission product from the glucoside, but an impurity. Moreover, tannase is formed only in the case of cultures on substrata containing tannin. Its optimum temperature is about 67° C., and it splits up tannates as well as phenyl- and methyl-salicylate. The same enzyme is probably concerned in the formation of gallic acid in opium fermentation, CALMETTE (II.) stating that *Asp. niger* plays the chief part in the fission of tannin during that process, and also in the inversion of the sugar into dextrose during this prolonged fermentation, which occupies ten to twelve months. Both the dextrose and dextrin are oxidised into calcium oxalate, without the alkaloids being affected. *Asp. niger* is well known as a fungus preferring acid substrata (solutions of organic acids), on which it thrives; and since it also occurs on gall-nuts and extracts of these, its spontaneous appearance in tannin- and opium-fermentation is easily accounted for. It is certain that spontaneous green, vegetative growths of *Penicillium species*, that need further investigation, play a chief part in the fission of tannin. Moreover, the gallic acid fermentation of gall-nut tannin was ascribed to "organised ferments," even anterior to van Tieghem. Thus LAROQUE (I.) in 1850 credited this "ferment" with the power of exciting alcoholic fermentation, without discriminating between the various organisms. On the other hand, ROBIQUET (I.) in 1852 brought about the same fermentation by means of an enzyme (pectase) in gall-nuts, which enzyme was also said to convert pectose into pectin. In comparison with these opinions, the views afterwards expressed by van Tieghem may be regarded as reactionary. Nevertheless the gradual modification of the ideas held on this point is not without interest.

An enzyme capable of saponifying fat was isolated in small quantity from "*Penic. glaucum*" by CAMUS (III.) in 1897. The extract from *Asp. niger* gave only a very weak effect in the hands of the same worker (IV.), though this fungus will grow luxuriantly on certain fats (*e.g.*, olive oil in presence of nutrient salts). GÉRARD (II.) in 1897 demonstrated the occurrence of lipase in *Penicillium*, by means of the method elaborated by HARRIOT and CAMUS (I.), but found that the emulsin of this fungus cannot split up fats. LAXA (II.) in 1902 showed that triturating the hyphæ of *Penicillium* liberates an enzyme capable of splitting up butter fat with considerable energy. According to BREMER (I.), a gradual effect of fission is produced on cotton-seed oil by *Aspergillus* species (*A. glaucus* and *A. flavus*). LABORDE (VI.) states that *Allescheria* (*Eurotiopsis*) also will split up oil and butter fat energetically, with formation of acid. Lipase was stated by GARNIER (II.) to occur in the cultures of *Asp. fumigatus A. flavus*, *A. glaucus*, *A. niger*, *A. nidulans*, and especially in *A. versicolor*. The fission of

fats in the sludge of clarifying tanks has already been dealt with (*see* p. 47, vol. ii.).

§ 293. Formation of Alcohol.

With a single exception none of the *Aspergillaceæ* excites an appreciable alcoholic fermentation. It is true that several species have been credited with forming alcohol; but, even where this has been shown beyond doubt, the quantity produced is insignificant. Sanguineti (I.), states that *Asp. oryzæ* forms alcohol from saccharose, starch and dextrin (up to 4 per cent. by weight in ten days), so that—assuming that, as reported, this organism can form 20 grms. of alcohol from 50 grms. of saccharose in the time mentioned—this fungus should be regarded as an important exciter of fermentation. Sanguineti's isolated experiments, however, need further confirmation. According to Pasteur (XXV.) *Asp. glaucus* forms about 1 per cent. of carbon dioxide and alcohol when submerged in wort—though not when exposed to the air—the mycelia separating into a number of rounded cells; and similar minute quantities of alcohol are said to be produced, in culture liquids, by *Penic. glaucum*. Gosio (VII.) reports in similar fashion with regard to *Penic. brevicaule*. Doubts, whether justified or not, have been thrown on this alleged capacity in the case of *Asp. glaucus*, *Asp. niger* and *Penic. glaucum;* though the statement of Elfving (I.), that he found up to 4.2 per cent. by weight of alcohol in cultures of "*Penic. glaucum*," is rather strange. One cannot reject off-hand the possibility of this substance being present in fungus cultures to a larger extent than is now believed; and the matter requires closer attention. Perhaps the alcohol has hitherto escaped notice owing to the circumstance that the vegetation of certain species readily decomposes ethyl alcohol (*see also* p. 58, vol. ii.). For instance, according to Laborde, *Eurotiopsis* can decompose up to 10 per cent. and *Asp. niger* (according to Duclaux) up to 6–8 per cent., whilst this substance, in the form of a 3 per cent. solution (when accompanied by mineral food-stuffs), is a suitable nutrient material for both *Asp. niger* and *Penic. glaucum*—compare Wehmer (V.) and Coupin (I.). Hence, when—as is usually the case—these organisms cannot be grown in a restricted supply of air, a rapid oxidation of the alcohol—sufficient to prevent accumulation—must be reckoned with. Mazé (II.) regards alcohol as a normal intermediate product of the decomposition of sugar by micro-organisms, and supported this opinion by experiments with *Allescheria Gayoni* (*Eurotiopsis*) in 1902.

This fungus, in fact, constitutes, according to Laborde (VI.), the single exception already mentioned. It excites normal fermentation in solutions of dextrose, lævulose, maltose and lactose—subsequent to enzymatic fission in the case of the two last, succinic acid and glycerine being formed in addition to alcohol and carbon dioxide. A restricted supply of oxygen is an essential condition, but none of these fungi will survive the total exclusion of that gas. There is no production of spherical yeast, as in many of the *Mucorineæ*, the submerged mycelium retaining its appearance unchanged. From 100 grms. of sugar Laborde obtained, on the average, 46.4 grms of alcohol, 44.4 grms. of carbon dioxide, 2.3 grms. of succinic acid, and 1.8 grm. of glycerine, with an increase of 4–5 grms. in the weight of the fungus (total 94.9 grms.). In the case of the first two sugars, this result corresponds to about 2 grms. less than by fermentation with *Saccharomycetes*, the latter furnishing Pasteur with 48.6 grms. of alcohol, 46.8 grms. of carbon dioxide, 3.2 grms. of glycerine, 0.6 grm. of succinic acid and 1.2 grm. of yeast (total, 100.4 grms.). The appearance of the fermenting fungus closely resembles that of *Mucorineæ* under the same conditions, the submerged mycelium developed from the sowing being quickly interspersed with large bubbles of gas, and also exhibiting a tendency to pass over into surface vegeta-

tion. In about six weeks the alcohol produced amounted to upwards of 8 per cent. A 14 per cent. solution of invert sugar was attenuated down to 2 per cent. of sugar in sixteen days, the lævulose disappearing comparatively quickly. Inverted lactose gave a more sluggish fermentation, 4–5 per cent. of alcohol being formed. Galactose by itself was more difficult to ferment, the process ceasing on 2–3 per cent. of alcohol being formed. Maltose (1–2 per cent. of alcohol) and lactose (2–3 per cent. of alcohol) behaved in a similar way; and their fission anterior to fermentation is difficult to determine. In presence of air, the fungus readily consumes alcohol, even when—as already mentioned—10 per cent. is added to the culture liquid. Nearly the whole of the sugar in a 10 per cent. solution disappears, within twelve days, when in contact with the surface vegetation of the fungus at 25° C., without more than 0.2 per cent. of alcohol being detectable. There is nothing remarkable in this, in view of the aforesaid fact (reported by Wehmer) that sowings of conidia of *Asp. niger* and *Penic. glaucum* on a 3 per cent. solution of alcohol (as the sole organic food-stuff) and inorganic nutrient salts, will develop to complete vegetative coatings; whilst, according to Duclaux, these cultures of *Asp. niger* will also decompose 6–8 per cent. of alcohol.

§ 294. The Degradation of Proteids and their Derivatives.

The property of liquefying gelatin is so general among the filamentous fungi, including the *Aspergillaceæ*, that only the exceptions are really of interest. The rapidity of this liquefaction—and probably sometimes also the time of its inception—depends largely on special conditions (the concentration and reaction of the gelatin, the presence or absence of certain substances, the temperature, &c.). Even the same species does not always behave in the same way, and therefore the appraisement of its diagnostic value is probably on a par with the case of bacteria (*see* vol. i. p. 230), though the feature possesses a certain importance in any event. The liquefactive power of *Penic. glaucum* seems to have been first investigated by A. Hansen (I.) in 1889, and that of *Asp. niger* by Bourquelot (XII.) in 1894. A tentative comparison, with streak cultures in 10 per cent. wort gelatin at 15° C., by Wehmer (XII.) showed that *Asp. glaucus* and *A. fumigatus* liquefy a gelatin very slowly, the results not being appreciable until several weeks have elapsed; whereas about half the gelatin was liquefied in ten days by *A. niger*, *A. oryzæ*, *A. candidus*, *A. minimus*, *A. novus*, *A. ostianus*, *Penic. glaucum*, *P. luteum*, *P. italicum*, and *P. olivaceum*; and, according to Wehmer (XVII.), *Asp. clavatus*, *A. flavus*, *A. Wentii*, and *A. giganteus* act with equal promptness. Schäffer (IV.) has also published the results of experiments in the same direction, and with about the same fungi. If well-defined conditions be maintained, the results may be utilised for diagnostic purposes; at any rate, the secretion of the liquefactive enzyme is not retarded by the presence of sugar. Only scanty information is yet available as to the nature of the proteolytic enzyme or enzymes. That of *Penic. glaucum* was extracted by A. Hansen (I.) in 1889 from the vegetations by means of glycerine. The solution converted neutral gelatin into glutopeptone more rapidly than acid gelatin, whether sugar were present or not. Isolation by precipitation with alcohol was found to be impracticable, the resulting precipitate being inoperative. Possibly the quantity obtained was too minute, since the experiments showed that the substance is actually excreted by the hyphæ into the substratum, and acts at considerable distances, as well as through an artificial collodion film.

Stoll (I.) carried out a series of comparative experiments on the proteolytic power of *Penicillium* species, the influence of the reaction of the medium being also observed, normal, acid (acidified with normal sulphuric acid), and alkaline

(with normal caustic soda) gelatin and sugar gelatin (containing 2 per cent. of dextrose) being employed at a uniform temperature. *Penic. brevicaule* liquefied alkaline gelatin more quickly than the acid sample (4–6 days), but did not liquefy sugar gelatin, though this latter was very gradually liquefied by *Penic. glaucum* under the ordinary experimental conditions. Increased additions of alkali or acid seemed to favour the action in this latter case, whereas further additions of sugar had a contrary effect. *Penic. olivaceum* liquefied the same acid and alkaline gelatin only after nearly four weeks, whilst sugar gelatin remained unaltered at the end of a fortnight. *Penic. italicum* also had no effect on sugar gelatin, though it acted on acid or alkaline gelatin after about a fortnight, the same behaviour being observed with *Penic. rubrum* and *Penic. purpurogenum*. Hence, with the exception of "*Penic. glaucum*," the addition of sugar prevented the liquefaction of gelatin (*see also* p. 46, vol. ii.). The previously mentioned experiments, and the observations of Malfitano, show that these results must not be taken, unconditionally, as generally applicable, other circumstances, such as the concentration of the gelatin, the presence of other nutrient substances, &c., having to be considered, since *Penic. brevicaule*, for example, is known to have a decided liquefying influence on 10 per cent. wort gelatin (*i.e.*, gelatin and sugar). Further particulars on the behaviour of four species of *Aspergillus* toward gelatin will be found in a recent work by Tiraboschi (II.).

The fact that an extract from *Asp. niger* soon dissolves fibrin and coagulated egg albumen, and also liquefies gelatin, was already reported by Bourquelot. Malfitano (I.), who was the first to investigate this point more fully, found that the method of nutrition was immaterial as regards the formation of the proteolytic enzyme ("protease"), this apparently diosmotising only after the death of the cell. It can be recovered by drying and grinding young and still living vegetative growths, and then extracting them with chloroform water, and using alcohol as a precipitant. The action of the enzyme is retarded by an acid reaction, neutrality being the most favourable condition and alkalinity highly prejudicial. Casein and uncoagulated albumen are also attacked, though less powerfully, whilst coagulated albumen and egg albumen are left intact. Milk casein, thrown down by the lab enzyme, is gradually dissolved. Though nothing certain is yet known about the final product of the reaction, this protease is apparently different from pepsin, pancreatin and papayin. Butkewitsch (I.) also occupied himself with the enzymatic proteolysis effected by the same fungus. According to Duclaux (VII.), "*Penicillium glaucum*" contains tryptic casease in addition to the lab enzyme (*see* vol. i. p. 186). The further degradation of protein by the fungi under consideration results finally in the formation of amino acids and ammonia. *Aspergillus niger*, however, as was shown by Wehmer (V.) in 1892, forms large quantities of ammonium oxalate in solutions of peptone, 5 grms. of peptone furnishing more than 2 grms. of calcium oxalate; and, according to Kosjatschenko (I.), it also produces from the protein of peas, tyrosin, leucin, histidin, arginin and lysin. On the other hand, according to Butkewitsch (I.), "*Penicillium glaucum*" seems to furnish chiefly amino acids (leucin and tyrosin), so that tryptic enzymes are apparently in question, as was proved by Saito (II.) by the formation of tryptophane in the case of nineteen species of fungi. Some practical importance also attaches to the question of the degradation of protein in the ripening of certain cheeses (Brie, Camembert and Roquefort) by species of *Penicillium*, on which point reference should be made to the labours of Roger, Epstein, Jensen and Thom.

The coagulation of milk is effected in 2–10 days by all the species examined on this point by Schäffer (IV.), viz., *Asp. niger*, *A. fumigatus*, *A. glaucus*, *A. Wentii*, *A. oryzæ* (2 days in this case), *Penic. glaucum* (in 3 days), *P. luteum*, *P. italicum*, and *P. rubrum*. These species also peptonised milk casein,

coagulated egg albumen, fibrin (except *Penic. glaucum* and *P. rubrum*), and vegetable casein. Teichert (I.) also pointed out that "*Penicillium glaucum*" has a decided degrading action on casein, and, according to Conn, Thom, Bosworth, Stocking and Issajeff (I.), both the technical *Penicillium* species of Roquefort and Camembert cheese (*P. Roquefort* and *P. Camembert*) also attack cheese by an excreted proteolytic enzyme. Lab enzyme was also found by Saito (II.) in *Asp. oryzæ*. Similarly, according to Swanoff, *Asp. niger* and "*Penic. glaucum*" contain an enzyme (nuclease) which splits up the nucleoproteids into xanthin bases and phosphoric acid.

Shibata (I.) states that *Asp. niger* produces an enzyme, or group of enzymes (amidases), furnishing ammonia, like urase. The triturated, dead mycelium acts on urea, biuret and certain acid amides (acetamide, oxamide), with formation of ammonia. On the other hand, urethane, guanidin, allantoin and uric acid remained intact, and the action on benzamide and asparagin was barely appreciable, whilst hippuric acid was split up into glycocoll and benzoic acid. Stoll (I.) also observed the formation of ammonia by *Penic. brevicaule* from ordinary gelatin. An unrecognisable species, *Asp. terricola*, is said by Wiley (I.) to be a powerful ammonia-former in soil; but the production of ammonia compounds from organic nitrogen compounds is not a specific characteristic.

§ 295. Colouring-matters, Poisons, Oxidations, &c.

On saccharine substrata containing traces of arsenic or arsenious acids and its salts, *Aspergillus glaucus*, "*Penicillium glaucum*," *Penic. brevicaule*, &c., liberate strong-smelling diethylarsine (*see* p. 37, vol. ii.). According to R. Schmidt (II.), "*Penic. glaucum*" and *Asp. flavus* liberate sulphuretted hydrogen from sulphates, &c., and arseniuretted hydrogen from solutions containing arsenic. Dubois (III.) states that *Penicillium* mycelia will precipitate basic copper carbonate (patina), from solutions containing copper, on to bronze. The frequently reported fixation of free nitrogen on the part of "*Penic. glaucum*" and *Asp. niger* by Berthelot (II.), Puriewitsch (VII.) and Saida (I.) need only be mentioned here (*see* vol. i. pp. 270, 271). Little is yet known as to the nature of the yellow, brown, and red colouring-matters produced by various species (*Asp. niger*, *A. glaucus*, *A. Ostianus*, *Penic. luteum*, &c.). According to Linossier (II.), that formed by *Asp. niger* ("Aspergillin") is an organic compound of iron; but this remains to be proved. Zukal (III.) states that the colouring-matter produced by *Penic. luteum* is a "fungus acid"; at any rate it is a substance soluble in alcohol and reprecipitable by water. A golden yellow pigment is said by Milburn (I.) to be produced by *Asp. niger* under certain conditions, in the form of a granular excretion from the aerial hyphæ. The alcoholic solution is decolorised by alkali, but not by acid, and the pigment is decomposed by light into a reddish brown substance, so that it is found only in cultures kept in the dark. Possibly the dark pigment of the conidia is formed therefrom by oxidation. R. Meissner (I.) carried out tests with the red-brown pigment of *Asp. medius* (probably synonymous with *Asp. glaucus*). The green conidial pigments produced in the vegetative growths of most *Aspergillaceæ* have not yet received attention. The pigment, soluble in alcohol, of *Asp. versicolor*, Vuill., varies between yellow-brown, orange, and red, according to the reaction of the nutrient solution; *see* Vuillemin (II.), and also Coupin and Friedel (I.) on this point. The dependence of the production of yellow to red colouring-matters on the composition of the substratum, especially as regards *Penicillium* species (*P. olivaceum*, *P. purpurogenum* and *P. rubrum*), has been mentioned by Stoll (I.).

The injuries set up by species that are pathogenic in plants and animals (*Asp. fumigatus*, *A. flavus*, *A. nidulans*, *Penicillium luteum*, *P. glaucum*,

P. italicum, *P. olivaceum*) are probably attributable to the production of definite poisons; but, in the case of those belonging to the second category—a list of which was compiled by Guéguen (III.)—no further particulars are yet available. J. Behrens (IX.) also failed to ascertain anything definite with regard to the active substance in the fungi which cause the rotting of fruit, but it is apparently not an enzyme and is non-volatile. A similar *rôle* is ascribed to free oxalic acid in the case of *Asp. niger*, which is said by Behrens (XVI.) to be dangerous to plant embryos. Lode (II.) failed to detect any poisonous substance in cultures of species that are pathogenic towards animals.

In several instances investigations have been made into the destructive action, exerted more particularly by vegetative growths, on readily oxidisable substances, such as organic acids and alcohols. This is related to the previously mentioned fact that oxalic acid, citric acid and ethyl alcohol are decomposed again by the fungi that have produced them (*Asp. niger*, *Penicillium*, *Citromyces*, *Allescheria*); but whether oxydases are concerned is still unknown. So far as the substances in question form suitable food-stuffs (tartaric acid, citric acid, lactic acid, &c.), this is nothing remarkable; but it is also exhibited, though to a small extent, in the case of the majority of such substances (acetic acid, butyric acid, propionic acid, &c.) when the degree of concentration is low. Laborde (VI.) reports that growths of *Allescheria* (*Eurotiopsis*) slowly decompose oxalic acid, malic acid (even when 2 per cent. is present), acetic acid (2 per cent.), propionic acid, butyric acid (0.8 per cent.), valeric acid (0.6 per cent.) and formic acid (to 1 per cent.), whereas inactive lactic acid was rapidly decomposed (even with 5 per cent.) without being split up into its active components (*see* vol. i. p. 178), and also methyl, propyl, butyl and amyl alcohol in small quantities. According to Duclaux (I.), growths of *Asp. niger* will decompose even 8–10 per cent. (?) of acetic acid, and also lactic acid and butyric acid (0.1–0.2 per cent., of which 0.5 per cent. is the smallest fatal dose. In presence of butyric acid or tartaric acid, the acetic acid was consumed more rapidly than either. Whether, as stated by Duclaux, *Asp. niger* in cultures free from bacterial infection is really capable of converting calcium butyrate into carbonate, and calcium lactate into carbonate and oxalate, is a point that needs closer examination, the mere statement being scarcely sufficient. Formic acid, in quantities up to 0.08–0.09 per cent, is decomposed by *Asp. niger* and *Penic. glaucum*, though—according to Duclaux—larger doses (0.12 per cent.) have an injurious effect, whilst, according to Wehmer, up to 10 per cent. of citric acid, tartaric acid and malic acid are decomposed by both fungi. In this connection, reference may be made to Pfeffer's (II.) reports on the selective affinity for nutrient substances (*see* vol. i. p. 36).

With regard to oxalic acid—the decomposition of which is mentioned in certain older reports by Warburg (I.) in the case of "*Penic. glaucum*," by Duclaux (XXI.), Werner and others—more accurate researches by Wehmer (XXVI., XXVII., and V.) have shown that 1 per cent. solutions are not attacked by these two organisms, whereas 0.2–0.5 per cent. solutions are completely, though slowly, decomposed. Soluble oxalates are decomposed with greater difficulty—and in the case of *Aspergillus* only under certain conditions—though with even a small growth of *Penicillium*, 1.5 grms. of potassium oxalate have been completely eliminated in sixty days. In all experiments of this kind, however, the nature of the food-stuff and the temperature require to be taken into consideration. It may be remarked, in conclusion, that *Asp. oryzæ* is credited by Aso (I.) with secreting an oxydase, whilst Pozzi-Escot (I.) states that the same fungus produces a reducing enzyme, which he calls "Jacquemase." Extracts of the vegetative growths of all the species examined by Schäffer (IV.) —*see* p. 270, vol. ii.—failed to give with acidified guaiacol solution the orange

precipitate stated by Bourquelot (IX.) to be characteristic for oxidising enzymes. Seven species, however, gave a positive reaction with guaiacol and hydrogen peroxide, among them being "*Penic. glaucum*," which, according to Grüss (I.), has no oxidising action. Saito (IV.) claims that *Asp. oryzæ* secretes catalase; and, according to Altenburg (I.), *Asp. niger* secretes an oxydase which liberates iodine from potassium iodide and was more closely examined by Raciborski (III.).

CHAPTER LVIII.

MYCOSPHÆRELLA TULASNEI AND SPHÆRULINA INTERMIXTA, OTHERWISE CLADOSPORIUM HERBARUM AND DEMATIUM PULLULANS.

By Prof. Dr. G. LINDAU,

Private Tutor at the Berlin University.

§ 296. Cladosporium Herbarum.

In the sub-order of the Sphæriaceæ (*see* p. 73, vol. ii.), species from each of two genera of the family of the *Mycosphærellaceæ* come under consideration here, whilst a third one will merely receive cursory mention. The last-named is the fungus—formerly known as *Læstadia Bidwellii*, and now as *Guignardia Bidwellii*—causing the black-rot disease in the vine, further particulars of which are to be found in Handbooks on Phytopathology, such as those of Viala (I.) and Sorauer (III.). The ascospores of this fungus are unicellular, but occasionally bicellular when ripe.

The very numerous species belonging to the genus *Mycosphærella* (formerly *Sphærella*), on the other hand, produce bicellular ascospores; and those of the genus *Sphærulina* (*see* p. 281, vol. ii.), even tricellular and polycellular ascospores. Of the first of these two genera only one species is of interest to us here, namely, *Mycosphærella Tulasnei*—on account of its conidial fructification—which, until recently, was still described under the name *Cladosporium herbarum*, given to it by H. F. Link, until its connection with the *Mycosphærellaceæ* was established by E. Janczewski (I.) in 1893. This worker succeeded in tracing the development of this new *Ascomyces* from the ascospores up to the production of ripe perithecia (*see* Fig. 184), and in obtaining, as a secondary fructification, the conidia (Fig. 185) with which alone we shall deal in the present paragraph.

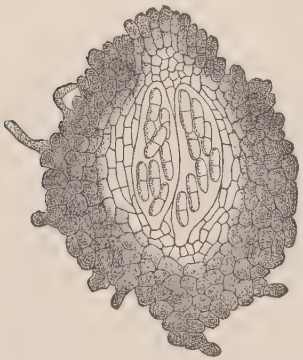


Fig. 184.—Mycosphærella Tulasnei (E. Jancz.). Longitudinal section through a perithecium. Magn. 325. (*After Janczewski.*)

Considered from the systematic standpoint, *Cladosporium herbarum* is probably a collective species, and appears to differ slightly in form under different conditions of cultivation. This explains why G. Fresenius (II.) and P. A. Saccardo (I.) described what seem to be different species, as *Penicillium cladosporioides* and *Hormodendron cladosporioides* respectively, whose proper place as forms of *Mycosphærella Tulasnei* was afterwards allotted them by Janczewski. This allocation has recently been questioned by W. Schostakowitsch (I.), who did not succeed in transforming the so-called *Hormodendron cladosporioides* into *Cladosporium*

herbarum. The assumption by Tulasne (II.) that *Cladosporium herbarum* belongs to the cycle of development of *Pleospora herbarum* was shown to be erroneous by the researches of Gibelli and Griffini (I.), by H. Bauke (I. and II.), and by F. G. Kohl (I.). Hence it may be assumed, in the present state of our knowledge, that, of the forms of *Cladosporium herbarum* hitherto described, one group has been recognised by Janczewski as belonging to the cycle of development of *Mycosphærella Tulasnei*, whilst others that have not yet been proved to so belong must continue in the meantime to figure as independent species in the literature. This remark applies, for instance, to that conidial fructification which was described as *Cladosporium herbarum* by Lopriore (I.). This species produces sclerotia which find a habitat on the husks of germinated and ungerminated wheat grains in the soil.

The progress of development of the conidial fructification of *Mycosphærella Tulasnei* described as *Cladosporium herbarum*, and first examined under the microscope by E. Loew (II.), is the exact antithesis to that of *Penicillium glaucum*. In the latter organism the outermost member of a conidial chain (the one furthest from the centre of growth) is the oldest and largest, so that the constriction of the several members proceeds from the periphery to the central point (basis) of the fungoid herbage, and is therefore basipetal (*see* p. 15, vol. ii.). The separate conidia are produced in succession immediately below the preceding ones on the conidiophore, which then, in order to counteract the resulting loss of length and to prepare for further constrictions, increases in length correspondingly. With *Cladosporium herbarum*, on the contrary, the faculty of direct constriction on the part of the conidiophore ceases with the production of the first conidium, and all the succeeding ones are formed from this latter—or from the daughter-cells produced in the meantime—by budding. In this case, therefore, the lowermost cell is the oldest, the top one being the youngest, so that the production of conidia proceeds from below (from the basis) upward (towards the apex), and is consequently basifugal, or, in other words, acropetalous. The budding capacity of a conidium is not confined to the formation of a single daughter-conidium, a second adjacent bud being oftentimes formed, which is capable of acting in the same way, and thus a richly branched formation ensues, as shown in the course of development in Fig. 186. The basipetal constriction of conidia by *Penicillium glaucum*, on the other hand, is naturally incapable of such a method of development, and is confined to the formation of a simple conidial chain.

Fig. 185.—Mycosphærella Tulasnei (E. Jancz.). Mycelial filament with conidia. (*After Janczewski.*) Magn. 250.

According to the observations of Janczewski (II.) a considerable degree of variation prevails in respect of the dimensions of the mycelium and the conidia of *Cladosporium herbarum*, a circumstance explaining the practice of classifying these various forms as different species, before this diversity was recognised.

Thus, the length of the ordinary ovoid conidia (Fig. 187, varies from 12 to 25 μ, and the breadth from 5 to 10 μ. The dimensions of the mycelial filaments vary accordingly, so that one worker may be confronted with a giant form,

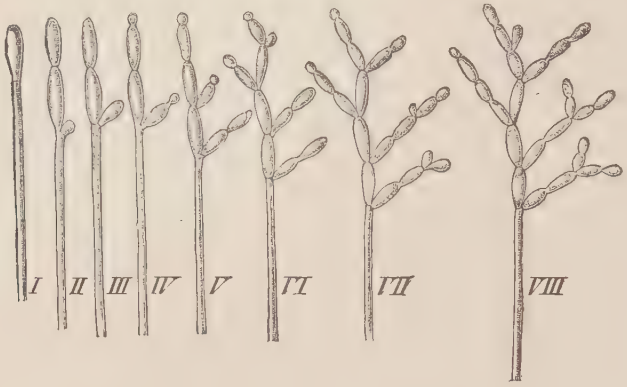


Fig. 186.—Cladosporium herbarum.

Conidiophores showing the successive formation of conidia during continuous observation on grape-juice ; *I*, commencement of constriction ; *II*, after 3 hours ; *III*, after a further $2\frac{1}{2}$ hours ; *IV*, after $10\frac{1}{4}$ hours longer ; *V*, after an additional 6 hours; *VI*, after a further $2\frac{1}{2}$ hours ; *VII*, $3\frac{1}{2}$ hours later ; *VIII*, still later. Magn. 300. (*After E. Loew.*)

whilst another may have a dwarf specimen. The number of septa within the conidia also varies with the age; two being present in one case, whilst another cell contains only one, and a third exhibits none at all. The external surface of the brown or olive-green membrane of the conidia may be covered with fine needles (crystals ?), though oftentimes it is smooth. On the basis of this characteristic, the systematist has elaborated various species of *Cladosporium ;* but in the present state of knowledge we cannot say whether these are specifically distinct forms or only caused by differences of environment.

Fig. 187.—Mycosphærella Tulasnei (E. Janez.).

Conidia from the mycelial filament shown in Fig. 185, two of them being asceptate, two with a single septum and one with two. Magn. 650. (*After Janczewski.*)

The above-described conidiophores are often found in black mildew on dead parts of plants, on damp cellar walls, casks and vats, the surfaces of these being covered with a herbage which is light olive-green when young, but gradually passes through olive-brown into dark brown. Simultaneously the cells thicken, become filled with drops of fatty oil, and, according to E. Laurent (VI.), also store up glycogen.

In practical fermentation, *Cladosporium herbarum* not infrequently makes its appearance as a source of damage, especially on cereal grains and on malt that is stored in a damp place. The hop plant, too, is occasionally infested and damaged by *Cladosporium herbarum*, particularly in damp weather or when the plants have been rendered susceptible to attack in consequence of other influences. In this case the fungus appears as an olive-green to brown growth on the under side of the leaves. The same fungus also seems to play some part in shed mouldiness in tobacco; and it is frequently noticeable in cellars. For this reason it is by no means surprising to find that

the fungus penetrates the corks of wines that are stored in bottle, and contributes to the production of corked flavour (*see* p. 238, vol. ii.) in such wines, as a result of its musty metabolic products. Particulars of this have been collected by J. Wortmann (III.). The fungus is also a frequent cause of damage in cheese dairies, where it plays a part in the blackening of the cheese.

The putrefaction of eggs is not always due to bacteria (*see* p, 167, vol. i.), but frequently to *Eumycetes*, the most active of these being *Cladosporium herbarum*, or, what is practically the same thing, *Hormodendron cladosporioides*. As long ago as 1864 it was shown by Mosler (II.) that uninjured eggs may be infected from the outside by *Penicillium glaucum* and *Mucor mucedo*. Zopf (X.) stated that Montagne cultivated *Dactylium ooyenum* from a rotten egg; and the same is reported of *Macrosporium verruculosum* by O. E. R. Zimmermann. The latter worker also observed *Torula ovicola*, *Penicillium glaucum*, *Stysanus stemonitis*, and its parasites, *Echinobotryum atrum*, and species of *Sporotrichum*. Finally, *Hormodendron cladosporioides* was frequently observed by Zopf (X.) in such eggs. According to the infection experiments carried out by Drutzu, conidia of the said fungus that have been accidentally or designedly placed on the unbroken shell of the egg germinate, penetrate the shell and internal membrane, and develop between the latter and the yolk to an agglomerated, gelatinous, dark brown mycelium, which gradually consumes the albumen; so that in certain cases none of this latter is left, the yolk being enveloped in a thick coat of fungus. On air gaining admission, in consequence of the gradual contraction and drying up of the contents of the egg, conidia are formed. Further particulars of the decomposition of eggs by *Cladosporium herbarum* have been furnished by Cerlese (III.) and Guéguen (IV.). A reliable means of preventing this incursion of aerobic *Eumycetes* is afforded by varnishing or liming the eggs while fresh.

According to a report by F. Rathgen (I.), a fungus was discovered in patina (*see* p. 275, vol. ii.) by L. Mond and G. Cuboni, and was named by them *Cladosporium aeris*. From comparative experiments they were obliged to conclude that this fungus contributes to the destruction of bronze.

§ 297. Dematium Pullulans.

On p. 278, vol. ii., it was stated that the genus *Sphærulina*, belonging to the family *Mycosphærellaceæ* (a sub-order of the *Sphæriaceæ*), is distinguishable from the allied genus *Mycosphærella* by its multicellular ascospores. Of that genus only a single species, *Sphærulina intermixta*, is of interest to the fermentation physiologist. The small perithecia of this fungus are found on withered rose-twigs; and one of the asci from the ascospores of this is shown, with its eight multicellular spores, in Fig. 188. When ripe, the asci are forced out of the perithecium, the ascospores being then liberated in consequence of the swelling up of the wall of the ascus. On finding themselves on a suitable substratum, they swell up immediately, develop longitudinal and transverse septa in their several cells, and also produce daughter cells by budding, so that a multicellular colony is soon formed. The daughter cells also detach themselves from the mass, and develop in the same way to a daughter colony. Still more commonly the cell-reproduction proceeds chiefly in a single direction, and then furnishes filamentous chains of cells, as shown at 5 in Fig. 188. These chains bear a close resemblance to the *Hyphomyces* described, under the name *Dematium pullulans*, by A. de Bary (III.), and occurring in nature in many kinds of black mildew, on sweet fruits, and on moribund parts of plants. Brefeld (X.) then recognised this *Hyphomyces* as identical with the above conidial fructification,

and consequently allocated *Dematium pullulans* to the morphological cycle of *Sphærulina intermixta*. Nevertheless, according to Alb. Klöcker and H. Schiönning (VII.), an unmistakable difference exists between them. The only way in which this question could be finally settled would be by inducing the *Hyphomyces* to develop perithecia, which no one has yet succeeded in doing. However, for the present, *Dematium pullulans* may be classed as a (still undetermined) species allied to *Sphærulina intermixta*, even if it cannot be regarded

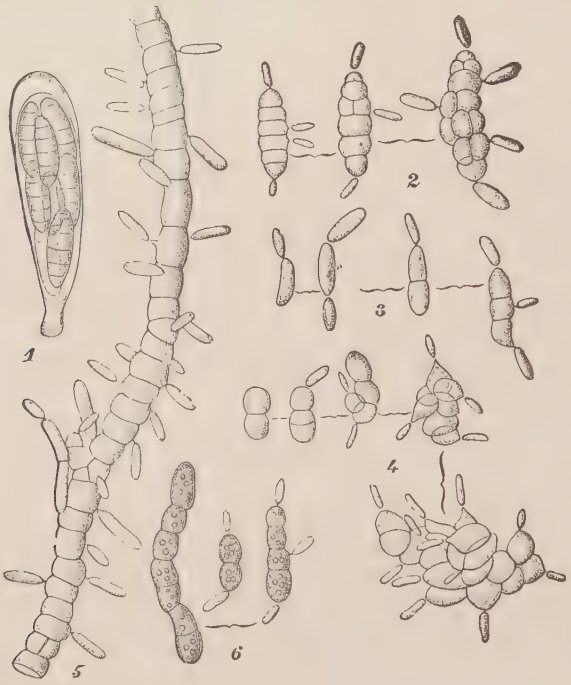


Fig. 188.—Sphærulina intermixta, Brefeld.

1. An ascus with its eight ripe spores.
2. Three ascospores swelling up and germinating.
3. Budding cells separated from the above, in course of reproduction.
4. Colonies from same.
5. Filamentous chain of cells, Dematium form.
6. Gemmæ.

Magn. 350. (*After Brefeld.*)

as forming part of the morphological cycle of the latter. The identification of species in this group is still in a very defective condition. The morphological similarity is very great, not only between *Dematium pullulans* and the conidial fructification of *Sphærulina intermixta*, but also between both and the conidial fructifications of the *Dothidea ribesia* and *D. puccinioides* described by Brefeld, the *Fumago salicina* examined by Zopf (XI.)—forming the chief constituent of true smut—*Cladosporium herbarum*, and others. Undoubtedly, however, *Dematium pullulans* stands nearest to *Sphærulina intermixta*, and its consideration in this place is therefore justified. On its account alone has mention been made of the said *Ascomycetes*, the ascospores of which, on the other hand, are developed

only outside liquids, and possess little interest to the fermentation physiologist beyond the developmental history sketched above. The views of earlier workers, who sought to establish a connection between *Dematium* and the true yeasts (*see* p. 79, vol. ii.) are entirely erroneous.

Consequently, the form known as *Dematium pullulans* alone constitutes the subject of the following lines. For exhaustive investigations into its structure we are more particularly indebted to E. Loew (V.).

If one of the yeast-like buds (conidia) of *Dematium pullulans* be placed in a suitable environment, it grows to an extensive mycelium, the several members of which throw up numerous ellipsoidal conidia in turn. According to the researches of W. Schostakowitsch (I.) on the influence of external conditions on the formation of the budding cells (*see* p. 17, vol. ii.), however, these conidia are not produced when the mycelium is made to grow in a strong solution of grape sugar or saccharose. The limit in this respect was determined by O. von Skerst (I.) as about 50 per cent. A temperature of 30°–31° C. has a similar restrictive influence; though by gradual habituation to progressively increasing temperatures it is possible to obtain at last a culture the mycelium of which will produce conidia at 30° C. At 50°–55° C., however, the mycelium is killed, after an exposure varying with its age.

On air being freely admitted to the nutrient solution, the hitherto slender, colourless cells of the mycelium are transformed into short, protuberant forms (gemmæ, *see* pp. 18, 19, vol. ii.), the membrane of which thickens and acquires an olive-green to brown tone. The depth of this colour depends, according to O. von Skerst (I.), on the richness of the nutrient solution, and increases therewith. At the same time an abundance of fat collects in the cells in the form of drops, which increase in size and are rendered noticeable by their power of refracting light and consequent lustre. In fact, this peculiarity renders the drops of oil liable to be confounded with endogenous spores—a point on which warnings have been issued by E. Laurent (VII.) and afterwards by O. Seiter (I.). This was also probably the cause of the mistaken opinion formed by Johan-Olsen (I.), whose error was corrected by Alb. Klöcker and H. Schiönning (IV.). In some cases the gemmæ subsequently develop a longitudinal septum, due to the transverse division of the mycelial cells. At a still later period the external layers of the thick cell wall sometimes become mucinous to such an extent as to render the nutrient liquid viscous (*see* vol. i. pp. 219–221). In this manner a culture of *Dematium pullulans* (grown, for instance, in beer wort) will develop into a greenish brown to dark green, thin, but viscous, film, resembling paper, on the surface of the liquid, whilst a deposit of yeast-like conidia and conidial cultures collects at the bottom.

According as the gemmæ are well nourished or the reverse, they either develop into a mycelium from which lateral buds are separated by constriction, or they produce these buds direct.

Dematium pullulans also affords an example of the cell fusion already referred to on p. 5, vol. ii., by the coalescence of two adjoining cells of the same mycelium, one of them penetrating the other. In *Dematium pullulans* more particularly—which, as already stated, exhibits a marked tendency to the formation of budding cells by constriction—there gradually develops, within the invaded cell, or host, a varying number of approximately ellipsoidal cells, which are in turn capable of reproduction by budding. In this way, under favourable conditions, a filament may become filled with cells which an observer unfamiliar with its method of origin may readily mistake for an ascospore (*see* Fig. 189). Such an erroneous impression has already been produced in the minds of several workers: for instance, O. Johan-Olsen (I.) in the case of a fungus he named *Dematium casei*,

and also Alfr. Jörgensen and Fr. Weleminsky (I.) with *Dematium pullulans* itself. We are indebted to A. Klöcker and H. Schiönning (IV. and VII.) for the correct interpretation of the phenomenon. This correction destroys the corresponding erroneous classification of *Dematium pullulans* with the group of the *Exoascece* or similar low *Ascomycetes*.

Fig. 189.—Dematium pullulans.

Filaments *a*, *b*, and *c* contain fused adjacent cells, some of which are constricting conidia. In *c* the septa at both ends of the cell have developed conidia; *f* might be mistaken for a quadricellular sporangium. In *d* a conidium is budding like yeast. Magn. 500. (*After Klöcker and Schiönning.*)

As a result of its frequent occurrence on straw, and therefore in the atmospheric dust in cowsheds, *Dematium pullulans* is likely to be often found in milk; and it has actually been detected in that liquid on many occasions by Adametz (III. and IV.). When the milk is curdled, a larger or smaller proportion of the fungus content passes into the curd. Its mode of action in this case re-

quires elucidation, and an attempt to explain it was made by O. Johan-Olsen (I.). This worker discovered in Norwegian "gammelost" (*see* p. 62, vol. ii.), a hyphomyces which he termed *Dematium casei*, regarding it as allied to *D. pullulans;* and, in a second communication (II.), he stated that the fungus caused the very bitter flavour of the cheese in question. A repetition of his examination, however, by Klöcker and Schiönning, led to the discovery that the fungus does not belong to the genus *Dematium*, but is more closely allied to *Monilia* or *Oidium*.

SECTION XVI.

GENERAL MORPHOLOGY, PHYSIOLOGY, AND CLASSIFICATION OF TECHNICALLY IMPORTANT BUDDING FUNGI OF THE GROUP "FUNGI IMPERFECTI."

CHAPTER LIX.

TORULACEÆ, PINK YEASTS, AND BLACK YEASTS.

By Prof. Dr. H. WILL.

Head of the Physiological Department of the Munich Scientific Station of Brewing.

§ 298. Historical, Delimitation, Derivation.

The name *Torula* was applied by Pasteur (XXVII.) in 1862 to a group of fungi, which reproduce by budding, like yeast, and are devoid of a typical mycelium. Plate III. of that authority's *Etudes sur la Bière* gives a very good and characteristic illustration of two species of *Torula* with globular cells, so that no doubt can exist in the case of at least one of the groups of budding fungi that were included by Pasteur under the generic name of *Torula*.

Pasteur also gave six pictures of other forms of *Torula*, of which those he showed in Figs. 1 and 2 of the above work also exhibited globular cells, whereas, as was pointed out by Hansen (LIII.), the one shown in Fig. 6 resembles the budding cells of *Dematium*. It is therefore doubtful whether this form should be allocated to the *Torulaceæ* at all or forms a separate group, though similar, pointed cells occur in many *Torula* forms with mixed cells. Figs. 3 and 6 of the said Plate, on the other hand, depict species in which thin, elongated budding cells appear in association with those of more contracted form (down to globular cells), and these exhibit certain analogies with the budding fungi now allocated to the genus *Mycoderma*. Pasteur also logically classed *Mycoderma vini* with the *Torulaceæ*, the difference between this fungus and the other *Torula* forms consisting, in his opinion, merely in the special structure of the cells and in a greasiness which causes the cells to adhere superficially. This feature, however, is not a strong one.

The species shown in Fig. 5 of the Plate in question is morphologically similar to an ordinary yeast (*Saccharomyces*), but probably also belongs to the *Torulaceæ*.

Pasteur himself expressed doubt as to whether all the forms depicted represented an equal number of species, and showed (as illustrated in his Figs. 1 and 2) that the elongated cells could also produce those of the small, globular type. He held the opinion that different varieties could be obtained from a *Torula* species with mixed cells, by sowing the different cell forms. Although he adduced no proofs in support of this, it is nevertheless correct, the more so because—as we now know—there are *Torula* forms which, under the influence of a certain

environment, reproduce almost exclusively in the form of small, more or less globular, cells, the elongated cells being comparatively small in number, whilst under other conditions these latter cells become more prominent.

Pasteur mentions, as a chief characteristic of the *Torula* forms depicted in his Figs. 1–6, that, like *Mycoderma*, they are unable to set up alcoholic fermentation.

Although Pasteur was able to characterise a series of the chief representatives of the *Torulaceæ* with a fair amount of certainty, notwithstanding that he was not in possession of absolutely pure cultures, the line of demarcation between them and the *Saccharomycetes* is uncertain and imperfect; and among them may be found *Saccharomycetes* with weak powers of fermentation.

It was not until the researches of Hansen (XXIV.) that the two could be more effectually separated. This worker applied the name *Torula* to budding fungi which produce neither endospores nor typical mould vegetations, thus differentiating them from the *Saccharomycetes* on the one hand and from *Monilia*, *Dematium* and other budding *Hyphomycetes* on the other.

By this definition Hansen greatly restricted the morphological circle of the *Torulaceæ*, allocating to the latter only such species as produce cells of a more or less globular shape, although some of his species form elongated, sausage-shaped cells in film vegetations. In any event, this limitation excludes from the *Torulaceæ* all the asporogenic budding fungi resembling *Mycoderma*, as also *Mycoderma* itself. Moreover, whilst Pasteur's *Torulaceæ* set up merely a very weak fermentation, or none at all, the species classified by Hansen exhibit all gradations in this respect, a few of them setting up a fairly vigorous fermentation. Consequently, Hansen's *Torula* species (*see* pp. 7, 8, vol. ii.) cannot be unconditionally united in one group with those of Pasteur.

The faculty of sporulation excludes from the *Torulaceæ* all such of the *Saccharomycetes* as—like the species *Torulaspora Delbrückii* (*see* p. 209, vol. ii.) of P. Linder (XXXVI.), and another species discovered by him in the mucinous exudation from oak-trees—occasionally form strictly globular cells, each containing a large drop of oil, like those of typical *Torulaceæ*.

Whilst it is true that the diagnostic characteristics set up by Hansen define the *Torulaceæ* more sharply than was done by Pasteur, recent investigations have brought to light difficulties in the way of this delimitation. The *Torulaceæ* are separated from the *Saccharomycetes* by a merely negative characteristic, namely, the absence of sporulation, whereas in other respects they have many points in common, both morphologically and physiologically, with the latter, as also with other asporogenic groups, such as *Mycoderma*. Hansen himself, however, showed that some of the *Saccharomycetes* lose their power of sporulation (*see* p. 191 *et seq.* vol. ii.) under certain conditions of treatment; and though the occurrence of such variations in the natural state has not yet been demonstrated, it is not impossible. In the absence of any information as to their origin, such forms would have to be grouped with the *Torulaceæ*. In the case of many *Saccharomycetes*, sporulation is also known to be of very difficult and rare occurrence, and one that evidently depends on certain well-defined but as yet unrecognised conditions.

The present writer's own researches have shown the existence of typical *Torulaceæ* incapable of setting up fermentation with the ordinary kinds of sugar, and therefore coinciding in this respect with those of Pasteur. He has also become acquainted with forms which, as mentioned above, produce almost exclusively small, more or less globular cells under certain conditions of cultivation, these cells exhibiting all the characteristics of the *Torulaceæ*, and being rarely accompanied by elongated forms. Under different conditions, however, the latter forms become more frequent, and thus constitute an intermediate link

with the species forming *pastorianus* cells (*see* p. 84, vol. ii.) in addition to those of oval and globular shape and mycelial agglomerations of same. They also possess fermentative properties, thus differing from the *Mycoderma* species.

On the basis of his own investigations, the writer, in contrast with Hansen, enlarges the morphological circle of the *Torulaceæ* so as to include species like those characterised by Pasteur.

Consequently, the following lines will treat not merely of such budding fungi as have not yet been observed to sporulate—and comprising those forming exclusively more or less rounded or oval cells, with or without the power of exciting fermentation (first sub-group)—but also of those producing mixed cells, developing interchangeably, but distinguished from the *Mycoderma* species by their fermentative power (second sub-group). The *Monilia* are excluded by the possession of a septate typical mycelium (*see* chapter lxii.). No fundamental reason exists for excluding the so-called pink yeast (or some of them at any rate) from the *Torulaceæ*, many of them, so far as is known, having many points of resemblance, morphologically, with the *Torulaceæ*, although they have not been studied with any thoroughness. Pigmentation cannot, at least, be regarded as a sufficient reason for their separation, since the researches of Kossowicz (I.), confirmed by R. Schander (I.), show that several of the *Saccharomycetes* which are colourless under ordinary conditions develop a red colouring-matter in certain circumstances, notably in presence of salts of magnesium. On the other hand, some of the typical forms of *Torula* occasionally assume a pink coloration only under certain conditions of growth, such as in films on nutrient liquids and in slant colonies. Pigmentation is by no means a constant feature. Nevertheless, the so-called pink yeasts and other coloured budding fungi will, for practical reasons, be dealt with separately in the present chapter under § 301.

The opinion that the *Torulaceæ* are only stages in the development of other fungi has already been expressed by Hansen (LIII.). It is known that the conidia of certain *Ustilagineæ* (*see* p. 79, vol. ii.) are able to maintain an independent existence, by budding, in suitable nutrient solutions. Budding cells are also met with in various groups of fungi; and possibly similar biological conditions may give rise to the same or similar external phenomena. The *Torula* group is not a natural one, and is merely of a temporary character.

E. Klein and M. Gordon (I.) claimed to have traced the origin of a pathogenic pink yeast to *Puccinia suaveolens*. On the other hand, R. Meissner (II.), in comparing his six species of mucinous yeasts (*see* p. 129, vol. ii.) with the budding cells of *Exoascus* spores, with which they seemed to have some connection, established an important point of difference between them and the budding cells of *Exoascus deformans*. It may also be remarked that Laurent (VIII.) has stated that the budding forms of *Cladosporium herbarum* (*see* p. 280, vol. ii.) are transformed into a pink yeast by insolation. In addition, Winkler (II.) claimed that *Mucor* spores, under certain culture methods, furnished "yeast cells" that were asporogenic, for which reason he proposed to group them, *pro tem.*, with the *Torulaceæ*. Agreeable though the idea may be that these organisms are merely budding forms of the conidia or spores of higher fungi, reports in this connection require to be very critically examined.

In treating of the asporogenic budding fungi mentioned in the literature, under the generic name of *Torula*, or more generally referred to as yeast, white yeast, &c., it is often difficult, nay impossible, to decide if they belong to the group under consideration. On the one hand, it must be remembered that the name *Torula* has been, and is still, applied at different times to very different organisms. Originally implying *Hyphomycetes* with conidia arranged in wreaths, with simple or branched chains, it was used by Turpin in 1838 to denote beer

yeast (*Saccharomyces cerevisiæ*), which he named *Torula cerevisiæ*. Cohn even applied the name *Torula* to the wreathed chains formed by the *Micrococcus* bacteria. At present the *Torulæ* form a sub-group of the *Dematiaceæ*: compare A. Engler and K. Prantl (I.). The species belonging to this sub-group, as, for example, *Torula monilioides*, Corda, found by F. Ludwig (III.) in the mucinous exudations of trees (*see* p. 100, vol. ii.), are outside the morphological circle.

Saccharomycetes greatly resembling certain forms of *Torula* are also known, as stated above. The statement "sporulation could not be detected" affords no guarantee that sporulation would not occur under certain conditions. In the opinion of many observers, all monocellular fungi that reproduce by budding, especially when they also set up fermentation, are yeasts, that is to say, *Saccharomycetes*; and they apply the name *Saccharomyces* to all such species described by them. The descriptions are mostly very imperfect, and it is difficult to decide with any degree of certainty, from the isolated characteristic peculiarities, whether the budding fungi under consideration belong to the *Torula* group or not. Matters are little better in the delimitation of the species that have been more closely examined; and any attempt to arrange them into a system has small prospect of success at present.

For the same reason it is difficult to decide whether the comparatively small group of (presumably asporogenic) budding fungi exhibiting the characteristic property of being able to ferment with sugars (*see* p. 296, vol. ii.) belongs to the *Torulaceæ*. Of this group the following members have been described: the so-called *Sacch. galactocola*, by Pirotta and Riboni (II.); one species by Duclaux (XXIII.); the so-called *Sacch. lactis*, by Adametz (XI.); the so-called *Sacch. Kefyr* and *Sacch. tyrocola*, by Beijerinck (XX. and XXV.); several species by Weigmann (XI.) and Grotenfelt (II. and III.); *Torula Duclauxi*, by E. Kayser (IV.) as well as the so-called *Sacch. lactis*, Adametz, and a species isolated from milk; one species by L. C. Mix (I.); *Lactomyces inflans caseigrana* by Adametz (XII.), in collaboration with W. Winkler, and also by Nicola Bochicchio (I.); the so-called *Sacch. Kefyr*, by Freudenreich (XI.); *Torula amara* by A. Kalanthar (I.); by Harrison (II.); and by O. Jensen (III.) and P. Mazé (I.), who also included in his examination the *Torula Duclauxi*, the so-called, *Sacch. lactis*, Adametz, and the species described by Kayser. The so-called *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, were subjected to a careful examination by Heinze and Cohn (I.). These two last-named species undoubtedly belong to the *Torulaceæ*, *Sacch. lactis* to the second sub-group and *Sacch. tyrocola* to the first sub-group, so that they ought, correctly, to be named *Torula lactis* and *Torula tyrocola* respectively. On the other hand, it is questionable whether the kephir yeasts belong to this category, though in other respects they should be classed with the *Torulaceæ*; since, according to the concordant statements of Adametz, Freudenreich and Heinze (III.), they are, of themselves, incapable of fermenting milk sugar. It is highly probable that the kephir granules contain a variety of budding fungi, and the so-called *Sacch. Kefyr*, Beijerinck, is apparently not always present. On the other hand, it is very likely that Boersch (I.), who attributes sporulation to the so-called *Sacch. Kefyr*, was examining a species that is not always contained in kephir granules; for, as far as the writer is aware, the bulk of the budding fungi in these granules do not sporulate. It is doubtful whether all the various forms described represent so many distinct species. Comparative researches by E. Kayser have shown that *Torula Duclauxi*, the so-called *Sacch. lactis*, Adametz, and the species isolated from milk by himself, possess very divergent and constant chemico-physiological properties. *Sacch. Kefyr*, Beijerinck, closely resembles *Sacch. lactis*, Adametz, whilst *Sacch. tyrocola* is probably identical with *Torula Duclauxi*. According to

the researches of Heinze and Cohn, little doubt can exist as to the separate identity of *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck.

§ 299.—Occurrence, Dissemination, and Morphology of the Torulaceæ.

The *Torulaceæ* are very widely disseminated. The frequency of their occurrence in the air depends, however, on certain conditions, chiefly on the way in which the ground is planted, the fruits of vineyards and orchards in particular affording the most favourable environment. Hansen (II.) found them in the air of the open country under fruit trees between July and November, most abundantly in September, whereas they were absent in May, June, and December; and this report is confirmed by the investigations of the writer. According to Hansen (II.), their normal winter habitat, like that of the *Saccharomycetes*, is the soil.

The *Torulaceæ* also find a home on field and garden fruits, and indeed on plants of all kinds; and they seem to find a suitable environment both during the decay of these fruits and during the technical processes for preserving same, such as the pickling of gherkins and beans, and the fermentation of sauerkraut.

Possibly the yeasts found in the fermentation of tobacco and tea also belong to the *Torulaceæ*.

They accompany the food into the stomach, and are found there in the human subject during complaints of that organ (fermentation and distension).

During the plague of *Lipara monacha* caterpillars in Bavaria, the intestinal tract of these insects was discovered to be occasionally packed with the cells of various *Torulaceæ*.

These organisms penetrate all kinds of organic substances and develop therein, frequently to a surprising extent. For instance, the thick white to whitish yellow coatings found on stored sausages consist sometimes of these fungi exclusively. Milk, butter and cheese also afford a favourable environment, and they occasionally develop abundantly in bread.

The numerous biological investigations of water, for brewing purposes in particular, have shown that river water is often very rich in budding fungi belonging to the morphological circle of the *Torulaceæ*. Budding fungi are also found in sea water, especially in northern regions; and some of these organisms are evidently *Torulaceæ*.

Establishments where dairying and fermentation industries of all kinds are carried on form a chief habitat of these budding fungi; and they develop enormously not merely in the raw materials and products, but also in the air of all the rooms, in the walls of the fermentation- and store-rooms, and on the utensils employed.

The form and dimensions of the cells vary considerably, more particularly in members of the second sub-group. Cells of one and the same species, grown in a given nutrient medium, often vary but slightly, though sometimes to a not inconsiderable extent, so that purely globular forms are accompanied by oval and more or less elongated types of *pastorianus* cells, especially in very old cultures. In species producing globular cells on the average, a few sausage-shaped or irregularly formed daughter-cells are developed; and even those resembling *Sacch. apiculatus* are regularly observed in certain species. The shape of the cells is influenced by the reaction and composition of the nutrient medium; and above all by the presence of certain sugars.

The cell dimensions of members of the first group vary within wide limits; and the cells of certain species may almost be mistaken for those of globular bacteria.

A very remarkable form that appears regularly, not only in the *Torulaceæ*, but also (though to a smaller extent) in the *Saccharomycetes*, is that of giant cells (*see* p. 85, vol. ii.), the dimensions of which greatly exceed the average size. These giant cells, occurring regularly (so far as observation has extended) in the second sub-group of the *Torulaceæ*, are often found in a decrepit condition. Whether they are abnormal, or cells endowed with certain physiological functions, cannot be decided at present.

Still greater variety exists in the form and size of the cells of the second sub-group with mixed cells. Cells agreeing in form and size with those of the first sub-group are accompanied by club-shaped, sausage-shaped and filamentous cells of all grades. Other species develop very thin and graceful cells; and the spindle-shaped cell, tapering off at both ends, is a not infrequent form. Filamentous cells, measuring up to 40 μ in length and 2 μ in diameter, have been observed. Chains of elongated cells frequently develop numerous rounded and oval cells on the ends of the individual members (in giant colonies).

As already mentioned, the appearance of this elongated cell form is connected in part with certain conditions of environment, so that, for example, the organism inside the nutrient liquid produces mainly or entirely the more compact cell forms, whilst the elongated forms are produced on the surface, as in the film vegetations of *Saccharomycetes*.

In giant colonies of many *Torulaceæ*, on the other hand, the superficial cells are chiefly globular or oval, whilst the under side exhibits numerous elongated, sausage-shaped cells, penetrating for long distances into the gelatin. Other forms, again, develop both within and upon the nutrient liquid, simultaneously with mixed cells.

The cell integument of *Torulaceæ* is of an even more highly diversified character than in the case of the *Saccharomycetes*. It is mostly strong, and in some cases of typical species attains a considerable thickness that is apparently associated with stratification. Still more frequent than in the *Saccharomycetes* (*see* p. 106, vol. ii.) is the phenomenon of sloughing the outer layer of skin. These extremely thick-skinned cells, which are found in nearly all cultures, are possibly resting forms (Chlamydospores). Conversely, the cell integument of many other *Torula* species is very delicate. Sometimes, as in the species depicted by P. Lindner (XXXVII.), and the mucinous yeasts of Meissner (II.), the cell wall develops an almost imperceptible mucous layer, whilst in other species a gelatinous network is clearly visible (*see* p. 130, vol. ii.) in the film vegetations on nutrient liquids, and occasionally the cultures transform the entire nutrient liquid into a tough gelatinous mass.

The cartilaginous film vegetations of certain close-growing species on nutrient liquids may be attributed to a peculiar condition of the cell integument. Species that, like *Mycoderma* and *Willia*, produce superficial films very rapidly, imprison air between the cells, a peculiarity favouring the assumption that the cell integument is of a greasy nature, like that of *Mycoderma*. No reports have been published dealing specially with the chemical composition of the cell integument of *Torulaceæ*.

Apart from isolated inclusions, the cell contents, as a rule, have only slight refractive power and remain pale, agreeing in this respect with *Mycoderma* and contrasting with the *Saccharomycetes*. While the cells are young, the contents are homogeneous, but afterwards turn cloudy and frothy; numerous small vacuoles appear, to give place subsequently to a single one (in globular or oval cells) or several (in elongated cells). The contents of older cells are occasionally crumbly and finely granulated, or a number of highly refractive granules appear. The number of these, however, is usually limited, the highly refractive inclusions forming a very characteristic element of the cell contents.

This applies particularly to the typical *Torulaceæ*, the species of the first sub-group.

As a rule, the globular cells contain an oily particle which seems to have been regarded by some authors as a nucleus. It is barely visible in submerged growing cells with homogeneous contents, and comes into prominence only on the appearance and growth of vacuoles, especially when the cells in film vegetations come into contact with the air. Even when the vacuoles have attained considerable dimensions, and the plasma has diminished to a mere stratum lining the cell wall, the oil particle remains coated with a layer of plasma. Usually globular, it is not infrequently flattened in appearance. The presence of an oil particle in the lactose yeasts characterises them as belonging to the *Torula* group. Raum (III.) differentiated one of these in kephir yeast by staining and assumed it to be a *Torula* form in the sense defined by Hansen. The size of the particle increases with the age of the cell and by contact of the latter with air, although, so far as observation goes, it remains small in some species, so that the size of the oil particle may serve as a means for the characterisation of species. The globular and oval cells of some species contain two or more oil particles; and these have probably been confounded with spores in many instances. The elongated cells of the second sub-group of *Torulaceæ* also contain oil particles, distributed in the same manner as those in the cells of *Mycoderma*. Nevertheless they may also be lacking in species with mixed cells, in the rounded and oval cells of which they occur regularly.

Crystalline bodies in the vacuoles (*see* p. 111, vol. ii.) form a highly characteristic inclusion in *Torula* cells. Of regular occurrence in a few species, they appear to be lacking in others with morphologically similar cells. Accordingly, they might serve as a diagnostic feature, in the same way as the varying number of oil particles in the globular cells.

Very old cells of typical *Torulaceæ* frequently contain, like those of the *Saccharomycetes*, a single large globule that is partly of a fatty nature. According to Lindner (XXXVI*b*) small fatty drops are of frequent occurrence in the small-cell species of *Torula*, even under normal conditions, the cells being mostly highly refractive, with a greenish tinge. *Torula pulcherrima* develops large, highly refractive globules.

Glycogen was absent from very few of the species examined by the writer, when grown in beer wort or in neutral yeast-water with 6 per cent. of saccharose. The production of glycogen was also observed by Meissner in certain of his mucinous yeasts. The intensity of the reaction varies considerably, but is generally faint, the most decided result being obtained with a species of high fermenting power. According to Heinze and Cohn, glycogen is formed by *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, to the same extent as by *Saccharomycetes*, and especially so in young cultures on acid wort gelatin. The red-brown coloration with iodine is given by the *Torulaceæ*, either in the plasma or the vacuoles; in the latter case it may extend over the whole contents of the vacuole, or be restricted to globular inclusions of varying dimensions.

With regard to the nucleus, the only reliable communication available is that of Guilliermond (V.), Beijerinck's report on *Sacch. Kefyr* leaving it doubtful whether he has not mistaken oil particles for nuclei. Other authors, who may be credited with a full acquaintance with morphological conditions, have asserted that a nucleus can be clearly discerned in cases where the oil particle is apparently alone in question.

The budding of the globular cells may take place at any point on the parent cell, sometimes occurring simultaneously in several places (coronation). The young generations form single, unbranched chaplets; and the order of budding is similar to that of the *Saccharomycetes* producing branched bud chains. Like

the *Saccharomycetes*, too, the members of these chains either adhere firmly together, so as to form chains of considerable length, or they separate readily into short lengths of 3–4 cells each. Acid nutrient media stimulate certain lactose yeasts to form extended chains, especially when the degree of acidity is high. A few species also branch extensively, even in distilled water. Not infrequently one is able to observe phenomena that approximate more closely to germination than budding, the cell bulging out in one place with a very broad basis and bursting at the same time, whereupon the growing daughter-cell becomes separated from the parent by a broad septum. The production of abnormal cells is frequently observed in budding.

The elongated cells of the second sub-group form either extensive chains of buds, or long mycelial rows with insignificant lateral branchings through shorter or longer cells; or again, they may produce a large number of globular *Torula* cells.

The giant colonies of the second sub-group with elongated cells are in some cases of very handsome appearance. The surface exhibits mesenteric folds of varying dimensions, though these folds are not always formed in presence of the elongated cells. In this respect, therefore, the giant colonies differ from those of most *Saccharomycetes*, but resemble those of *Willia* and *Mycoderma*, though differing from the *Monilia* species.

The giant colonies of the first sub-group, on the other hand, are usually more or less flat, with slightly dished edges, and exhibiting, at most, faint radial stripings, with numerous smooth or warty excrescences on the surface. These excrescences form a general characteristic of the giant colonies of this first sub-group, and are not confined to the *Torula* form isolated by M. Hartmann (I). from a dried yeast purchased in Java. Consequently, the specific name, *colliculosa*, applied to this *Torula* does not by any means characterise this species. Moreover, the fact stated by Hartmann, that these excrescences are composed of large cells, is not confined merely to this species.

In many forms the surface of the giant colonies bristles with numerous tufts. The nature of the nutrient medium has no great influence on the form of the giant colonies, the character and colour of which, moreover, are highly diversified. In many cases the colour is characteristic of the species: pale pink, yellow or yellow-brown, both in the film vegetations and in the giant colonies; though sometimes it is confined to, or attains its greatest intensity in, the latter, which thereby acquire increased diagnostic importance. Mostly, the colonies are colourless. They may be mucinous, gelatinous, or more or less dry, dull, semi-matt, or shining like cut glass or mother-of-pearl. Some species produce giant colonies of a waxy character or resembling enamel.

§ 300. Physiology and Chemistry of the Torulaceæ.

Reproduction in liquid nutrient media, like that on solid substrata, depends on the composition, reaction and concentration, as also on temperature and other external conditions, but primarily on the species itself. In a large number of species compared by the writer, the most favourable development took place in neutral yeast water containing 6 per cent. of saccharose, next in order coming the cultures in hopped and unhopped beer wort, and those in saccharine yeast water with an addition of 0.5 per cent. of peptone. Even a small quantity of peptone has great influence on the development, though asparagin also forms a good source of nitrogen. According to Beijerinck (XIX. and XXV.) and J. Schuurmans-Stekhoven (I.), *Saccb. Kefyr* also assimilates succinic acid; and growth is likewise stimulated by ethyl alcohol. Hayduck's nutrient solution is the least suitable food-stuff for the *Torulaceæ*. The *Torula* species examined by

the writer also grew well in beer, provided the liquid was not too deep. Meissner's mucinous yeasts throve in Raulin's solution, but less favourable results were obtained with E. Laurent's nutrient solution. All the writer's *Torula* species developed in milk, and in some cases produced a cheesy smell, whilst in isolated instances the milk was coagulated. Among the lactose yeasts which also thrive in this medium, *Lactomyces inflans caseigrana* alone produces coagulation without any important formation of acid. The coagulum is partly reliquefied. Certain of the mucinous species develop very slowly indeed in all the nutrient media examined, growth proceeding exclusively at the bottom of the vessel at first, whereas other species of the same sub-group reproduce at once and very rapidly, as films on the surface.

The production of a film occurs sooner or later with all the species hitherto examined by myself. Several of them, chiefly from the first sub-group, cover even alcoholic nutrient liquids with a film by the end of twenty-four hours, and develop, like *Mycoderma*, principally on the surface. The external similarity to *Mycoderma* species is the greater when the dry, dull grey films assume, like the latter, mesenteric folds in the course of further development. In certain cases the film remains smooth and delicate. The production of film vegetation takes a long time with some species, and occasionally nothing more than a ring is formed (*see* p. 88, vol. ii.), even at high temperatures. The films then exhibit a moist gloss, resembling that of *Saccharomycetes*, and are occasionally of a thick, mucinous character. Strongly developed films may become coloured (lemon-yellow, rose-red, leather-brown, olive-green).

During development in nutrient liquids, a variety of phenomena characteristic of the species are observed. A cloudiness may set in at first, to subsequently disappear with the formation of pulverulent, flocculent, agglomerate, yeasty, solid or mucinous, ropy sediments; or, as in the case of *Sacch. lactis*, the cloudiness may persist. In other instances the liquid remains perfectly limpid. Two species impart a decided lemon-yellow colour to saccharine yeast water. Other nutrient liquids, such as beer wort and must (*see* p. 163, vol. ii.), are decolorised to a greater or smaller extent. This has been established in the case of beer wort by L. van den Hulle and H. van Laer (II.), Will (XXXI.) and P. Lindner (XXXVII.); and for must by R. Meissner (II.). In Will's experiments, the highest degree of decoloration, determined by the method of C. J. Lintner (II.), was 0.6. Many *Torula* species, on the other hand, seem to darken the colour of beer wort, but whether this also applies to the *Torula Novæ Carlsbergiæ* of Grönlund (II.) must remain an open question owing to the unreliable method of colour determination employed by that worker.

The capacity of many *Torula* species for acclimatisation in highly concentrated nutrient liquids (*see* p. 167, vol. ii.), seems to be very extensive. Thus the writer found one species able to develop and produce a fairly brisk fermentation in a 76 per cent. malt extract. Wehmer's salt yeast remained capable of development for several months in herring pickle, representing a 24 per cent. solution of salt, whereas *Lactomyces inflans caseigrana*, Bochicchio, could not stand saturated solutions of salt for more than 30–40 minutes. An addition of 15 per cent. of common salt to the nutrient solution merely retarded the development of the salt yeast in question.

Acid nutrient solutions—fairly strong, *e.g.*, sauerkraut water, containing nearly 1 per cent. of lactic acid with some species—formed a more favourable medium than neutral for most of the species examined by myself. *Lactomyces inflans caseigrana*, Bochicchio, continued to vegetate in a broth containing 1–2 per cent. of lactic acid, and *Torula amara*, Harrison, even in one with 2.4 per cent. of that acid. A few of the species described by other workers, such as the *Torula* isolated from pine-apple by E. Kayser (V.) proved sensitive toward

acids, as did also the so-called *Sacch. lactis* and *Sacch. tyrocola*. All the species examined by Will (XXXII.) were able to stand direct treatment with a 4 per cent. solution of tartaric acid for forty-eight hours at 25° C. (*see* p. 180, vol. ii.).

Some species will even grow in alkaline media, Meissner's mucinous yeasts developing as quickly in alkaline Liebig's meat extract with sugar, as in wine must. On the other hand, the budding fungi—some of which at least must belong to the group now under consideration—discovered by O. Bail (I.) in decaying rhubarb leaves, disappear when the reaction of the leaf mass changes to neutral and alkaline. A series of budding fungi capable of fermenting lactose, isolated by Mazé, induced a far better fermentation in alkaline nutrient media than in those with an acid reaction. Probably the alkali fixes the acids that are liberated during fermentation and retards that process.

Carbon dioxide retarded the development of Meissner's mucinous yeast, without killing them, and the reproduction of these organisms decreases as the alcohol content (*see* p. 175, vol. ii.) of the nutrient liquid rises, ceasing when it reaches 9 per cent. in must, though the cells do not die. The power of resistance is a variable quantity. In the researches of Virgin (I.) reproduction ceased in the case of a species of *Torula*, when the alcohol in the grape-sugar broth reached 8.5 per cent., addition of ammonia causing rapid reproduction. Sulphur dioxide also influenced the activity and development of the said mucinous yeasts, about 0.1 per cent. being the limit for hindering development. Tannin restricted the growth and reproduction of the mucinous yeasts; and their resistance to acetic acid (*see* p. 180, vol. ii.) was very slight.

The temperature at which the known *Torulaceæ* continue to reproduce occurs between wide limits, nearly all of them growing even at 5°–6° C. The intensity of reproduction varies considerably, but is generally small at low temperatures, in which case, moreover, the character of the nutrient solution greatly influences development. Several of the species examined by myself remained for a month without reproduction, in pure yeast beer at about zero C., though some of them grew, if only to a small extent, in neutral saccharine yeast water; and a few also in hopped beer wort. With one of the species examined by Hansen (LV.), the minimum temperature was also 0.5° C. *Sacch. lactis*, Adametz, *Torula Duclauxi*, and the lactose-fermenting species isolated by E. Kayser from milk, will not adapt themselves to temperatures of about zero C. In the species examined by Will, the optimum temperature varied between 20° and 25° C.; whereas it lay between 25° and 30° C. for the Hartman's *Torula colliculosa*, *Sacch. lactis*, Adametz, and Duclaux's *Torula* and the lactose-fermenting species of Kayser. In the case of *Sacch. lactis*, Adametz, the optimum fermentation temperature was 37.5°–40° C., whilst *Sacch. tyrocola* preferred lower temperatures, viz., 23°–27° C. The optimum temperatures for development and fermentation do not always coincide. With *Torula colliculosa* the limit of growth was reached at 45° C., whereas Hansen found it to be 36°–37° C. for several of his *Torula* species, and in one case 38–39° C. formed the limit for several of the species examined by myself. *Lactomyces inflans caseigrana*, Bochicchio, grows very rapidly at 40° C.; but growth recedes at 45° C. and the fungus dies in a short time at 50°–60° C. The optimum growth temperature of *Torula amara*, Harrison, is 37° C., the limit being 48°–50° C. As in the case of the *Saccharomycetes*, a considerable divergence in the limits of budding temperature is observed among the *Torulaceæ*, a circumstance capable of affording valuable diagnostic indications.

Growth and reproduction are largely influenced by the admission of air. All the known *Torulaceæ* require free oxygen, a characteristic which certainly stands in causative connection with the predominating tendency of many species to grow on the surface of the liquid. This requirement, however, does not

extend so far as to necessitate direct contact with the air, growth proceeding also in fairly deep strata of liquid.

Numerous reports are available on the behaviour of the *Torulaceæ* towards the various kinds of sugar, *e.g.*, by E. C. Hansen (XLVI.), L. van den Hulle and H. van Laer (II.), E. Kayser (V.), C. Grönlund (II.), V. Peglion (II.), R. Meissner (II.), A. Kalanthar (I.), O. Bail (I.), M. Hartmann (II.), L. A. Rogers (I.), J. J. van Hest (I.), N. Hj. Claussen (I.), and chiefly by P. Lindner (XXXV.). The writer himself has also carried out numerous fermentation experiments with the *Torula* species he examined. The methods adopted by the various authors differed among themselves. P. Lindner, M. Hartmann and the writer employed the small-scale method (introduced by the first-named) in hollow-ground slides, with yeast water as the nutrient liquid. In any event the nutrient solution used plays a certain part in the fermentation.

Fermentative power is lacking in only a very few of the known species, such as the majority of Meissner's mucinous yeasts, and a few of the *Torula* forms examined by P. Lindner and the writer; but, apart from a few lactose-fermenting species, the *Torulaceæ* are not extensive alcohol-formers. Most of them ferment glucose, mannose, galactose and fructose with comparative readiness; maltose is fermented with difficulty, if at all, whilst the same species are able to split up the other sugars named into alcohol and carbon dioxide. In other cases, *e.g.*, *Torula colliculosa*, the maltose is fermented only by certain cells present in the warty excrescences of the giant colonies, whereas the cells growing in the flat portions of the colonies do not exhibit the least sign of fermentation in presence of maltose. Saccharose is fermented with vigour by a large number of species; but others cannot invert it, though able to reproduce at the expense of this sugar, as they do in the case of others they are unable to ferment. Lactose, trehalose, melibiose and melicitose are not split up into alcohol and carbon dioxide by the majority of species, and in a few cases the same applies to raffinose. *Torula Novæ Carlsbergiæ*, Grönlund, ferments dextrin.

On the other hand, a small group of *Torulaceæ*, including the species mentioned above, is characterised by the property of fermenting lactose, and consequently possesses high practical importance. So far as research has been pushed this group exhibits the same characteristic as the others, namely, that glucose, galactose and saccharose are fermented readily, maltose only with difficulty. When grown in beer wort, the non-sporulating, so-called *Sacch. pinophthorus melodus*, isolated by J. J. van Hest from spoilt beer, generates a gas that burns with a blue flame.

The fermentative power of a given species varies with the kind of sugar employed. Some of them, when grown along with yeast, are able to hinder the fermentation set up by the latter organism, probably in consequence of their transformation products. In some species the amount of alcohol produced is considerable. On the other hand, as shown by Heinze, the presence of 10 per cent. of alcohol in the nutrient liquid completely hinders the development of *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, even 5 per cent. being sufficient to suppress fermentation and reproduction almost entirely. Heinze and Cohn found the remarkable ratio of about 3 : 2 between alcohol and carbon dioxide with the last two lactose yeasts in meat-broth cultures containing lactose. Esters are also produced during the fermentation.

With regard to the enzymes of the *Torulaceæ*, little is known at present. Invertase appears to be excreted by many of them: compare Schuurmans-Stekhoven (I.) and E. Fischer (IV.). According to the researches of H. van Laer (VII.), inversion appears only in certain nutrient solutions. On the occurrence of lactase, which was questioned by Schuurmans-Stekhoven (I.) and E. von Freudenreich (XI.), *see* E. Fischer (IV.). Henneberg (V.) established

the presence of catalases, that decompose hydrogen peroxide, in living *Torula* cells.

Gelatin is liquefied by all the species hitherto examined, but the nature of the active enzyme remains undetermined. The *Lactomyces inflans caseigrana* of Bochicchio produces a lab enzyme and a tryptic ferment (compare p. 46, vol. ii.), and a fat-decomposing enzyme seems to be produced by several species. Nearly all the *Torula* species examined by myself are able to liberate sulphuretted hydrogen—sometimes to a very considerable extent—when sulphur is present in the nutrient solution (compare chap. lxvi.).

So far as information is available, the production of acid by *Torulaceæ* seems to be inferior to the acid consumption, though the amount produced is fairly considerable in some cases, notably by Kayser's pine-apple *Torula* (which produces acetic acid and small quantities of a higher fatty acid) and by Clausen's *Brettanomyces*. The *Torula* cultivated by Weigmann from bad butter produces about 3.6 per cent. by weight of butyric acid when grown in milk; but, in the researches of Heinze and Cohn, *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, seldom produced more than 0.3 per cent. of acid. Acid is also formed by Grönlund's *Torula Novæ Carlsbergiæ* and van Hest's *Sacch. pinophthorus melodus*, the amount varying with the kind of sugar. The nature of the acid in these cases is unknown. With the species examined by Will, on the other hand, the acidity of the beer wort varied, both the decrease (with one exception) and the increase being, however, inconsiderable. The development of one species changed the reaction of even strongly acid sauerkraut water to neutral in a month; and alkalinity ensued with a less acid medium. No regular connection could be detected between the diminution of acidity and the rapid production of a film growth on the surface of the nutrient liquid. Bail's rhubarb fungi consumed citric and tartaric acids.

The resistance of the *Torulaceæ* to high temperatures is fairly strong in a few species, but varies according to the species, the duration of exposure and the composition of the substratum. The age and physiological condition of the cells are also important factors. After being grown for eight days in wort, seven of the species examined by Will survived exposure to a temperature of 65° C. for half an hour, whilst the remainder, under the same conditions, were killed by a temperature of 60° C. In most cases the fatal temperature was the same for wort and water tests. Another *Torula*, reported by F. Schönfeld (XI.), possessed still greater powers of resistance, succumbing only when heated to 68°–75° C. in beer for an hour. On the other hand, van Hest's *Sacch. pinophthorus melodus* would not stand heating for five minutes at 65° C. The resistance of Meissner's mucinous yeasts terminates between 54.5° and 61° C., and all died when warmed at 45° C. for two hours. The fatal temperature in the case of the lactose-fermenting *Torula* species is 50° or 55° C. It is therefore evident that, under certain circumstances, the capacity for resisting heat may afford useful diagnostic indications.

Very low temperatures are also withstood well, Meissner's mucinous yeasts, for example, being found alive after exposure to – 22° C. for eight hours.

Several species will also stand desiccation, a fact already reported by Pasteur (XXVII.), who was able to convert his *Torula* forms into the dry state without loss of their powers of development. Meissner's mucinous yeasts were more susceptible, however, dying after five days' desiccation in the air. Harrison's *Torula amara* perished almost as quickly, in the dried state, at temperatures between 15° and 5° C., and Bochicchio's *Lactomyces inflans caseigrana* at 35° C.

Direct insolation had no destructive influence on Meissner's mucinous yeasts.

Considerable longevity is exhibited in liquids by some species, as in the case of the *Saccharomycetes*. Hansen (LI.) found living cells, capable of development, in cultures stored for sixteen years in a 10 per cent. solution of saccharose. In beer wort, some of the species perished in less than a year, whereas others were still living at the end of eight years. The mucinous yeasts found by Wortmann (XVII.) in twenty- and thirty-year-old wines (*see* p. 177, vol. ii.), exhibited great longevity.

So far as our knowledge goes at present, the *Torulaceæ* do not seem to be of any practical utility to man, or to play any important *rôle* in the economy of nature, though a number of species are capable of producing objectionable effects in the dairying and fermentation industries. A number of problems in which the action of *Torulaceæ* is concerned are still awaiting solution; and certain pathogenic budding fungi described by physicians belong to the *Torulaceæ*, though they need not be taken into consideration here.

According to the researches of Bail (I.), it is highly probable that certain *Torulaceæ* are causatively connected with the decay of many plants. The constant and abundant occurrence of budding fungi, also belonging to this group, in the excreted juices of preserved food-stuffs—*e.g.*, in herring pickle, the aqueous liquid of sauerkraut, and other food-stuffs and delicacies prepared in a similar manner by processes of fermentation—has raised the question whether these organisms are of importance in the preparation of the desired products or not; but no decision has yet been arrived at on this point.

A certain amount of importance attaches to several species in connection with the fermentation industries – especially in the preparation of beer and wine—on account of the maladies they give rise to in the products, in which they are able to reproduce themselves and live. The flavour of beer, for example, is greatly influenced in this way. The formation of aromatic products, exhibiting the flavour and smell of apples, seems to be a property of many *Torulaceæ*. It has often been asserted that the presence of *Torula* imparts a full, and even pappy, flavour to beer; and, in certain circumstances, this may well be the case. *Torula* species occur almost invariably in beer worts cooled and aerated in vessels that are not enclosed and protected from atmospheric infection. Experience teaches, however, that these organisms do not develop to any considerable extent, because, as shown by Will, most of them are suppressed entirely, or else greatly checked in their development, by the primary and secondary fermentations. It is a matter of experience that beer maladies due to *Torulaceæ* are extremely rare, and therefore these organisms cannot be regarded as injurious to beer in general. According to Will's researches, the addition of yeast to cultures containing certain mucinous species of *Torula* results in the so-called "boiling" fermentation (*see* p. 134, vol. ii.), in which the usual fine head on the liquid is replaced by a few very large bubbles. According to the concordant reports of N. Hj. Claussen (I.) and H. Seyffert (II.) certain species of *Torula* play an important part in the preparation of English beers. This group, known as *Brettanomyces*, is indispensable for the flavour and aroma developed in English beers by ethereal products formed during secondary fermentation.

Certain *Torula* species are valuable to the cattle-breeding mountaineers of Caucasia, as well as to the inhabitants of Armenia and the nomadic tribes of South-East and Southern Russia, since, in collaboration with certain bacteria, they serve in the preparation of important food-stuffs and delicacies, *e.g.*, kefir, koumiss, and mazun. Further particulars on this point are furnished by E. von Freudenreich (XI.) and A. Kalanthar (I.). Harrison's *Torula amara* imparts a disagreeable, bitter taste to milk and cheese; and, according to L. A. Rogers (II.), tinned butter is endangered by species of *Torula*.

§ 301. Red Yeasts and Black Yeasts.

Small as our knowledge is of the, usually colourless, *Torulaceæ* described in the preceding paragraphs of the present chapter, it is still less as regards the budding fungi that attract the eye by their more or less intense and variously shaded red colour. These are called by different authors "pink yeast" or "red yeast," some even classifying them with the genus *Saccharomyces*, though the majority do not form spores. The earliest attempt at a thorough investigation of these budding fungi was made, at a comparatively recent date, by F. A. Janssens and A. Mertens (I.), with a species described as "red *Torula*."

Red budding fungi have long been known. At first they were described by Fresenius (I.) under the name *Cryptococcus glutinis;* and, subsequently, Schröder and Cohn (I.) grouped similar organisms (termed "pink yeast" by Cohn) with the *Saccharomycetes*. *Cryptococcus glutinis*, Fresenius, and *Saccharomyces glutinis* are, however, apparently two different species. It was afterwards shown by Hansen (LII.) that the term *Cryptococcus glutinis* comprises a growth of several species, and that these cannot be properly assigned to the *Saccharomycetes*. One of the budding fungi examined by Hansen (LI. and LII.) is probably identical with Cohn's *Saccharomyces glutinis;* the second is a true *Saccharomyces;* whilst the third is characterised by the production of tubular buds, and is allied to *Cryptococcus glutinis*, Fresenius.

So far as Hansen (LIV.) and P. Lindner (XXXIV.) were able to re-examine the pink yeasts subsequently described by the Koch school and physicians generally, these species are incapable of sporulation. According to Lindner, Koch's pink yeast is identical with one of those drawn by Hansen (L.), the same bizarre outgrowths being exhibited by both. Sporulation is also lacking in Elfving's (II.) red budding fungus.

Red-coloured budding fungi are mentioned, and in part more fully described, by L. van den Hulle and H. van Laer (II.), who discovered one species in the Belgian beer known as Lambic (*see* vol. i. p. 195). The red *Torula* of Janssens and Mertens was isolated from the deposit in English bottled beer. M. Ward (VIII.) refers to *Cryptococcus glutinis* as an alien organism in ginger-beer yeast (*see* vol. i. p. 196). E. Kramer (III.) describes a red budding fungus taking part in the fermentation of must; and a pink yeast, found in fermenting must, is mentioned by V. Peglion (II.) and E. Kayser (XII.). A species occurring on milk and cheese, and named *Saccharomyces ruber* by R. Demme (I.), is regarded by him as the cause of gastric catarrh in children of tender age. It should be mentioned that A. Kalanthar isolated from mazun—a beverage of the kefir type, prepared in Armenia from the milk of buffaloes or goats—an orange-coloured budding fungus and a species the giant colonies of which were initially greenish grey, afterwards turning peach-red. Coloured budding fungi seem to be of common occurrence in milk and butter, Krüger (III.), for instance, having found in cheesy butter a budding fungus which he described as *Saccharomyces flava lactis* (*see* p. 208, vol. ii.); whilst R. Reinmann (I.) discovered pink yeast, along with other budding fungi, in butter.

A. Lasché (I.) isolated two species, *Mycoderma humuli* (from hop leaves) and *Mycoderma rubrum* (from an infected gelatin culture). B. Fischer and K. Brebeck (I.) found a pink yeast in the contents of the stomach of a patient suffering from gastric enlargement and fermentation; and another in the water of the open sea to the south of San Miguel, one of the Azores islands. C. Wehmer (XXXI.) also reports having found pink yeast in herring pickle. It is still doubtful whether certain species, such as the red yeast mentioned by A. P. Swan (I.), belong to the group now in question; J. C. Bay (III.) contests

their claim to be considered *Saccharomycetes*. Some doubt also attaches to the *Saccharomyces japonicus* and *Sacch. keiskeana* (*see* p. 176, vol. ii.) of K. Yabé (VI.). On the other hand, one of the species described by K. Golden and G. C. Ferris (I.) is said to be identical with *Saccharomyces glutinis;* and another species has been allocated to the *Mycoderma* group.

From all the reports, which could be amplified without difficulty, as to the occurrence of red-coloured budding fungi, it appears that these organisms are very common.

The arrangement of these diversified forms, the majority of which have not yet been thoroughly examined, into a system is still more difficult than in the case of the species comprised in the generic name *Torula*. It is equally difficult to decide whether certain of these forms are identical or not.

On the basis of their special method of vegetative reproduction, one of the species described by Hansen, as well as the *Mycoderma rubrum* and *Mycoderma humuli* of A. Laschè, the red *Torula* of Janssens and Mertens, Koch's pink yeast (according to P. Lindner), and the *Blastoderma salmonicolor* of Fischer and Brebeck, may be arranged in one group. A second group might include the forms with more or less globular cells, such as *Saccharomyces glutinis*, Cohn, one of the species described by Hansen, and several others. The latter, as producing the most pigment, might be united with the first sub-group of the *Torulaceæ*, with which they appear to have a good deal in common.

The colour of the cells is usually noticeable only when a large number are in juxtaposition. The shades of colour are numerous: pale red, rose-red, vermilion, coral, yellowish red, and salmon-red. Pigmentation seems dependent on certain conditions in many species, and in some occurs very late, so that it is not a constant feature. The intensity of the colour also varies, and is dependent, *inter alia*, on the reaction of the nutrient medium.

The cells vary in size and shape quite as much as with the *Torulaceæ*.

Like the *Torulaceæ*, too, highly refractive bodies that have undoubtedly often been mistaken for spores, occur in the cells, especially those of old cultures. In Janssens' and Mertens' red *Torula*, the bodies of this kind observed in the vacuoles resemble drops of oil, and are orange-coloured. They, however, consist largely of carotin, but do not appear to contain fat. A uniform reddish tinge is often visible in the vacuoles in old cultures of *Blastoderma salmonicolor*. In other respects there is no information available regarding the seat of the pigment in red budding fungi.

The nature of the pigment varies, being sometimes soluble in water, and disappearing under the influence of acids and alkalis; whereas, on the other hand, the red *Torula* of Janssens and Mertens gives a clear, deep red extract only with carbon disulphide.

According to reports by Laurent (X.), and also by Brault and Löper (I.), the red budding fungi produce glycogen.

Janssens and Mertens described a globular nucleus, with a nucleolus, in their red *Torula*.

In one and the same species budding may proceed in different ways. Sometimes it resembles the same process in the *Saccharomycetes*, with the modifications exhibited by the *Torulaceæ*. Raum (III.) found a parent-cell carrying up to five and more daughter-cells at the same time. In the case of a fixed cell of his species belonging to the second group, Hansen (LII.) observed a considerable number of new cells gradually formed at the same place.

In addition to this method of budding, the red yeasts of the first group also exhibit cell outgrowths in the form of "tubular buds" or "promycelia." Usually the oval cells throw out simple or branched, filamentous lateral growths, which, in association with the sterigmata, impart a strange appearance. The

budding of these "tubular buds" results in the production of rounded cells resembling conidia, or, in the case of *Blastoderma salmonicolor*, pear-shaped, plum-shaped or reniform cells. This type of germination forms a highly characteristic feature of the first group of red species, and, so far as is known, is not exhibited by any other group of budding fungi.

Films are produced by all species of red yeasts, and on the most divergent nutrient media, such as beer wort, beer (except in the case of *Mycoderma humuli*), milk, whey, &c. The films are partly smooth and mucinous, partly tough and greatly imbricated (*e.g.*, *Blastoderma salmonicolor*). The film of the red *Torula* of Janssens and Mertens is more strongly pigmented in the dark than in the light, and the cells are larger, but the resisting power is smaller. The film grown in the light resembles woolly felt, many of the filaments projecting above the surface of the liquid. Probably also hairs and tufts are formed, as in the case of *Monilia candida*, certain *Torula* species of the second sub-group, and occasionally also with *Saccharomycetes*. The cells in this case are smaller, but more resistant.

Reports on the giant colonies are few in number. P. Lindner (XXXVI.) has described those of two species, each of which exhibited a slight, mealy "bloom," a distinctive feature of the one being the production of delicate "white" aerial hyphæ. A notable feature is the formation of secondary colonies in plate and streak cultures, the more so because this phenomenon occurs in two species belonging to the group that germinates by promycelia and forms cells resembling conidia. Janssens and Mertens explain the phenomenon in the case of their red *Torula* by stating that the liquefaction of the gelatin is accompanied by the liberation of a gas, which forces the liquefied gelatin through the colonies and scatters it over the surface, a number of cells being carried off at the same time. Fischer and Brebeck, on the other hand, attribute the appearance of secondary colonies to the conidial cells being liberated by slight vibrations and then settling down in the vicinity of the original colonies.

The requirements of the red budding fungi in respect of organic nutrient materials have not been specially investigated, though it has been reported that starch paste forms a good medium for certain species. According to Hansen's (LIV.) researches, Elfving's red budding fungus will reproduce in purely inorganic media, a fairly strong light being essential. Hence, in this case at least, the red pigment plays an important part in the physiology of nutrition, though the possibility of saprophytic nutrition is not precluded. The red *Torula* of Janssens and Mertens is also influenced by light, and behaves like a green plant, respiration being also apparently more pronounced in the light than in the dark. The researches of Went (III.) with *Monilia sitophila*—in which the formation of carotin is dependent on light—indicate that the abundant production of carotin protects the enzymes of the fungus from strong light. Little is known as to the enzymes of the red budding fungi, though the action of catalase was observed by Henneberg (V.). Fermentation is absent, at least among the *Mycoderma*-like species of the second group, and appears to be only imperfectly developed in the members of the first group. E. Kramer's red budding fungus ferments dextrose, maltose and saccharose, which it previously inverts, but does not attack lactose. Fermentation for eight hours in sugar solution furnished 4.5 per cent. of alcohol, by volume, the solution at the same time acquiring an agreeable fruity aroma, indicating the formation of esters. The fermentation proceeded more actively in acid media than in those with an alkaline reaction, even 1.5 per cent. of tartaric acid being more stimulating than restrictive. Lindner (XXXV.) failed to obtain fermentation with any of the red yeasts examined. On the other hand, Kalanthar's greenish mazun yeast (p. 299, vol. ii.) possesses fermentative power.

Very little has been published on the behaviour of the red budding fungi toward acids. The red *Torula* of Janssens and Mertens produces only small quantities of acids, which are exclusively non-volatile.

The optimum temperature of growth is about 20° C., as with many species of *Torula*. The vital activity of Janssens' and Mertens' red *Torula* is impaired by a temperature of 30° C. A red *Torula*, isolated by Schmidt-Nielsen (I.) from the surface of the deep-water shrimp (*Pandalus borealis*) furnishes a luxuriant culture on potato slices in fifty to sixty days at zero C. E. Kayser's pink yeast withstands heating to 45° C. in the damp state.

So far as our knowledge of the red budding fungi extends at present, none of them possesses any great practical importance, except the case mentioned by Demme (*see* vol. ii. p. 299), though they are able, occasionally, to give rise to very unpleasant phenomena. Will (XXXIII.) cites an instance where such organisms coloured a whole batch of green malt red, their reproduction having apparently been greatly stimulated by peculiar circumstances. When dried, the malt turned a dirty brown colour, and the cured malt had an unsightly, discoloured appearance. The infection was traceable to the water used for steeping the barley. Beer wort is partially decolorised both by the pink yeast observed by L. van den Hulle and H. van Laer in Lambic, and also by the red *Torula* of Janssens and Mertens, the former yeast also imparting a sour taste to the wort. These properties, however, are of little practical importance, the red budding fungi and *Torula* species being suppressed by the rapidly multiplying and fermenting beer yeasts; and, even if they survive the fermentation process, the extent to which the water is decolorised by the beer yeast itself cannot be increased very much by the action of the red budding fungi.

Black yeast has also been reported upon occasionally. The fungus isolated by C. Marpmann (VI.) from milk, and termed *Saccharomyces niger* by him, forms round to oval cells, measuring 1.5–3 μ, and reproducing by budding. No mycelial filaments are produced in saccharine nutrient solutions. On gelatin, the fungus forms velvety black herbages, and in nutrient solutions black deposits. Saccharose and lactose are not fermented, though grape sugar is to a small extent. According to B. H. Buxton (I.), the fungus does not contain either diastase, maltase, invertase, lactase or inulase. Hansen (LIV.) has demonstrated that *Saccharomyces niger* does not sporulate, and is therefore no true *Saccharomyces*. According to him, the dark-coloured budding fungi belong to various species, all of them agreeing in being asporogenic and incapable of fermentation. He considers them to be, probably, budding forms of *Cladosporium* or *Fumago* species; and this view is supported by P. Lindner (XXXIV.) who states that, whilst the young cultures of black yeast grown in Koch's laboratory formed a pad consisting of bud cells, they subsequently developed into dark green herbages composed of hyphæ. Apparently Marpmann's black yeast differs from that of Koch. The *Torula nigra* of Guilliermond (IV.) grows luxuriantly on carrots, so that, twenty-four hours after sowing, the substratum is covered with a sticky, blackish green mass, composed entirely of oval and slightly elongated bud cells, held together by a mucinous mass exhibiting isolated black, solid particles. After a few days the less damp portions of the nutrient medium exhibit a thin mycelium, arising out of the black mass of yeast, and assuming the shape of a grey, matted felt. In Guilliermond's opinion, this fungus is probably allied to *Dematium*. P. Lindner (XXXVI*b*) mentions a black yeast, isolated by Zeidler, and having ellipsoidal cells, measuring 0.6 μ in length. On wort gelatin it develops with a damp surface and mesenteric folds, covered by a scanty growth of wool. Hansen states that black yeasts are not infrequently found in atmospheric dust, but he does not credit them with any practical importance. G. Grotenfelt (II. and III.) gives black yeast as the cause of blackening in cheese.

CHAPTER LX.

MYCODERMA.

By Prof. Dr. RICHARD MEISSNER,

Principal of the Royal Würtemberg Institute for Viticulture at Weinsberg.

§ 302.—Species of Mycoderma.

The film yeasts (*see* pp. 120 and 287, vol. ii.) comprise numerous species, of which comparatively few have, as yet, been thoroughly examined. They are all unicellular budding fungi, which reproduce either by budding and sporulation, or by budding only, and are therefore in part true *Saccharomycetes* of the genera *Pichia* and *Willia* (*see* pp. 211 and 213, vol. ii.), and in part non-*Saccharomycetes*. The latter may be divided into three groups, two of which belong to the *Torulaceæ* (p. 286, vol. ii.), the third comprising the various typical species of *Mycoderma*. These last alone will be treated in the present chapter to the exclusion of such species as were regarded as *Mycoderma* by earlier workers, but must be allocated to the pink yeasts or *Torulaceæ* on account of their fermentative power, oval cell form, or other peculiarities. These excluded forms comprise, for example: Heinze's *Mycoderma cucumerina*, Aderhold; the *Mycoderma* species mentioned by Lasché, *Myc. rubrum*, Lasché's *Myc. humuli*, Henneberg's two *Mycoderma* species, and the sporogenic film yeasts of Fischer and Brebeck. It may be mentioned here that the *Torulaceæ* and *Mycoderma* species have a number of properties in common; their distinguishing characteristics will be found on p. 286, vol. ii.

To the *Mycoderma* species belong, *inter alia*, a species of film yeast examined by Will (XIII.); certain film yeasts described by Meissner (XI.); others described by Hansen, A. Petersen, Grönlund, Jörgensen, Lindner, Prior, Bélohoubek, Kukla, Forti, Seifert, Lafar, Koch, Wortmann, E. Rist and J. Khoury, and others. Like the true wine yeasts, these various species of *Mycoderma* have their natural habitat in the soil, from whence, as shown by the researches of Hansen, Müller-Thurgau and Wortmann, they are conveyed to their appropriate nutrient solutions by insects, rain or wind.

Even as recently as 1871 we find Trécul expressing the view that proteid materials can change themselves into bacteria or direct into beer yeast, these again into *Mycoderma*, and the latter in turn into *Penicillium* (*see* p. 78, vol. ii.); and similar ideas are found in a treatise by Hoffmann (VII.) in 1869. At the same period, however, Adolf Mayer (X.) disputed the alleged genetic relation between yeast and *Mycoderma*, and between yeast and *Penicillium;* and Reess (IV.), in 1870, denied the identity of *Penicillium*, wine yeast and *Mycoderma*.

Moreover, the old assumption that the so-called *Sacch. Mycoderma* (*see* p. 200, vol. ii.), *Mycoderma vini* and *Myc. cerevisiæ* were one and the same species, has been disproved by the numerous researches of later workers. The paths by which this knowledge was attained are identical with those pursued by Hansen in establishing the existence of the different races of beer yeast, namely, by the

pure culture of the organisms, and by accurate morphological and physiological investigation.

There is no difficulty in obtaining material for the pure culture of different races of *Mycoderma*. Bottled wine, fruit wine, beer, &c., low in alcohol, is taken, half the contents of each bottle being poured out, and the remainder shaken up once or twice, after which the bottles are plugged with cotton-wool and are left to stand for several days at about 20° C. This treatment admits a sufficiency of oxygen to the bottles and liquids, so that the *Mycoderma* species and other organisms present therein are enabled to develop. Pure cultures of these organisms can then be prepared by the method recommended by Hansen (*see* p. 159 *et seq.*, vol. ii.).

The various races of *Mycoderma* can be differentiated by the size and shape of their cells, their rate of reproduction growth in giant, stab and streak cultures, the character of the superficial vegetation, and by the attendant phenomena of the same. Physiological examination also reveals differences between the various species that may also indicate racial peculiarities.

§ 303.—Form, Dimensions and Contents of Mycoderma Cells.

Attempts to identify single or mixed races of *Mycoderma* by microscopical examination alone meet with exactly the same difficulties as are encountered in the corresponding investigation of beer and wine yeasts, the problem being still further complicated by the fact that the development of *Mycoderma* in must or other nutrient media is accompanied by numerous morphological changes, whereas the true wine yeasts, for example, retain their form practically throughout such treatment. *Mycoderma* species, on the other hand, and especially while young, sometimes vary in form to such an extent that the observer might be led to think the cultures had become contaminated, if he had not, by constantly following the development of individual cells into chaplets, convinced himself of the morphological variability of the cells.

This phenomenon has been observed by different workers at various times. Winogradsky (XI.) attributes the variation, not merely to the specific nutrient medium, but more particularly to the greater or smaller supply of oxygen available for the growing vegetation. Thus, one and the same species will produce cells similar to those of yeast when oxygen is present, whereas, in the absence of that element, the growth is mycelial in character. It was also found by Will (XIII.) that the form of the *Mycoderma* species examined by him varied between wide limits, as did also the dimensions and contents of the cells; and Meissner (XI.) arrived at a similar result. In the races subjected to morphological examination by him, the largest cells measured 4.6 μ by 19.2 μ, those of the other races remaining below this level. According to Will (XIII.), the typical *Mycoderma* cells measure 8–11 μ in length (the mean being 9 μ), and 5 μ in breadth.

The cell form is pastorianous, with rounded edges (Figs. 190 and 191), though, as pointed out by P. Lindner (XXXI.) irregular half-moon and pear-shaped cells (Fig. 192) are also found now and then. Will (XIII.) gives precise data respecting the integument and contents of the cells. According to this worker, the young cells exhibit only faint refraction after their contents have been shrunk by the action of glycerine. The membrane is usually thinner than in yeast cells; and even in the slender cells, occurring regularly in older cultures, the integument is pale and seems to remain so always. On the other hand, *pace* Will (XIII.), the slightly oval, tough cells, appearing in older cultures, are distinguishable from the other cells by their strong membrane, bounded by a broad outline. Treatment with 1 per cent. osmic acid stained the cell wall dark brown, a result that

Meissner (XI.) did not succeed in obtaining in the case of wine *Mycoderma*. This behaviour on the part of the membrane led Will to conclude that substances behaving like fats or oils were stored up in or upon the membrane. The same worker (XI.) has also pointed out that the microscopical examination of *Mycoderma* cells has revealed the presence of individuals characterised by a certain lustre, accompanied by a bluish sheen. This peculiarity he ascribes chiefly to an enveloping stratum of air; and this conclusion was confirmed by Meissner

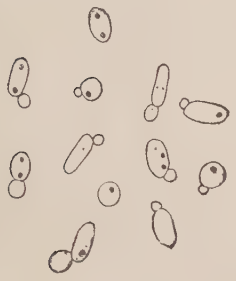


Fig. 190.

Mycoderma from Eltville red wine. Pastorianous and round cell forms. Magn. 600.

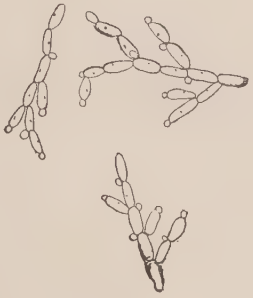


Fig. 191.

Mycoderma from bilberry wine (West Prussia). Pastorianous cell forms. Magn. 600.

Fig. 192.

Mycoderma from Rüdesheimer wine. Irregular cell forms. Magn. 600.

(XI.). The higher lustre of the cells may also be the result of their content of glycogen, which varies considerably in the individual cells of one and the same culture. As is the case with the cells of *Torulaceæ*, the contents of young *Mycoderma* cells have a low refractive power (*see* p. 291, vol. ii.), and consist, according to Will (XIII.) of a somewhat fluid substance that is stained by iodine. They must, therefore, contain a large proportion of water. The vacuoles, of which at first there are three, four or more in each cell, afterwards coalesce to form one or two. No highly refractive bodies (oil particles) can be observed in the very young cells; but when these cells are treated with iodine, deeply stained, dense granules become visible in the places where the refractive bodies are found in more mature cells. At the end of forty-eight hours these granules can be perceived without the aid of the reagent. By the third day they number from one to three, and are situated either at the ends of the cells, or one of them is in that position, whilst the other lies between two vacuoles at one side or in the middle. At the same time the vacuoles are more clearly visible, and exhibit a denser plasmal integument. At the end of forty-eight hours the cells will give a faint glycogen reaction with iodine. In aged cells crystalloids are gradually developed in the vacuoles, the oil particles come into being and attain considerable dimensions (2 μ in diameter). These latter are stained a blackish brown by osmic acid. According to Will the oil particles differ from those in old yeast cells by remaining unstained when treated with concentrated sulphuric acid, though, as with yeast cells, the oil is expelled from the cells on the addition of the acid. Further particulars on this point, and on the position, structure and subdivision of the cell nucleus, will be found in chapter xlvii.

§ 304.—Reproduction of Mycoderma in and upon Various Nutrient Media.

As already stated on p. 303, vol. ii., all the *Mycoderma* species reproduce by budding, which process has been described on p. 7, vol. ii. At present we will merely deal briefly with the formation of the bud aggregations, characteristic of the pastorianous *Mycoderma* cells, and easily distinguished from that occurring in the case of typical, oval yeast cells.

If a pastorianous *Mycoderma* cell be sown in a nutrient solution—*e.g.*, wort or grape-juice—and its development be followed under the microscope, it will be found to bud at one end in exactly the same manner as true yeast (*see* Fig. 193 *a*). As soon as the daughter cell (2) is completely formed, it buds (*b*) in the direction of its longitudinal axis, whilst the parent cell throws out a new daughter cell (3) at one side of the place whence the first cell (2) made its appearance. These new daughter cells in turn bud longitudinally (*c*), whilst their parent cells again develop lateral buds (*d*). Eventually the assemblage of buds assumes a form similar to that of a pine-tree, the central stem and lateral branches of which continue to grow in their initial direction, whilst branching out regularly every year. In the case of *Mycoderma* cells, this branching occurs about every two hours in a good nutrient solution; and as, when grown in wort or grape-juice, the assemblage of buds is not broken up by ascending bubbles of carbon dioxide, it frequently consists of hundreds of cells.

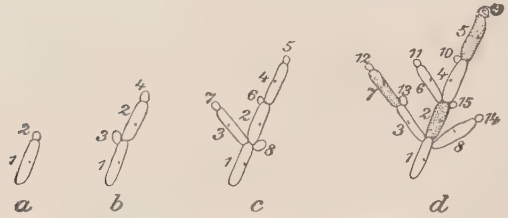


Fig. 193.—Aggregation of buds from Mycoderma. Cells 2, 5 and 7 are surrounded by an envelope of air. Magn. 600.

When a nutrient gelatin is employed as substratum, in which the cells are compelled to develop *in situ*, the cells commence to bud in the same manner; but since the daughter cells are unable to spread out uniformly, as is the case in or upon a liquid, a compact, spherical colony is formed, like those of beer or wine yeasts.

With regard to the giant colonies (*see* p. 291, vol. ii.), Lindner (XXXI.) rightly calls attention to the fact that their variability of form is greater in this case than with any other group of budding fungi. According to that worker, "In some cases the colonies form a dull grey to yellowish grey mass without any surface markings, or else veined, like leaves; in others a number of delicate and closely set concentric rings are exhibited, or wedge-shaped strata proceed from the centre of the colony, spreading out and usually assuming a dry, mealy appearance, or the entire surface becomes covered with innumerable fine or coarse wrinkles. In still other cases the colony takes the form of a hill, with a number of circular walls thrown up on the slopes; or it resembles a mountain peak with a number of dichotomous branches running down into the plain; or again, a miniature volcano, whose uniform slopes, covered with powdery white dust, exhibit a number of supplementary craters in the form of small, warty excrescences. Other colonies are broad and round, like cakes, with superficial fissures through which the pasty mass is exuding in the form of a round protuberance; or again, like a cake the substance of which has subsided along radial

lines." These descriptions of Lindner's, however, are only applicable to a few typical *Mycoderma* species. Meissner (XI.) classes the giant colonies of the *Mycoderma* races examined by him into four different types, according to their habit of growth. Type No. 1 comprises smooth colonies, the members of which differ in their lustre, the fluting at the edges, the extent to which they grow into and liquefy the gelatin medium, and in a smaller degree by their size and colour. Type No. 2 includes the circular, compact colonies, which differ in size and superficial markings. In type No. 3 the colonies are also compact, but exhibit more extensive markings on the surface (Fig. 194). In type No. 4 the colony is raised in the centre, sinking thence, with a concave slope, to a ring concentric with the original cell. Radial lines extend from this ring or wall to the edge of the colony; and between these lines, irregular depressions and excrescences (Fig. 195) can be observed. As regards the microscopical

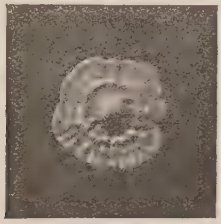


Fig. 194.—Giant colony of Mycoderma from Geisenheim currant juice. Nat. size.

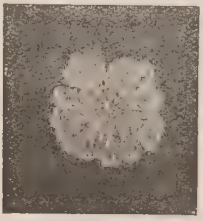


Fig. 195.—Giant colony of Mycoderma from Gau-Algesheim grape juice. Nat. size.

examination of the cells, it will be noticed—as shown by Will (XIII.) and Meissner (XI.)—that the cells around the edge of the colonies are larger than those nearer the centre.

In the case of stab cultures, Will (XIII.) showed that the species examined by him—which grew to a depth of 60 mm. into the gelatin—was able to bear a certain degree of deprivation of air. The streak cultures of Will's species failed to exhibit any characteristic features at the end of fifteen days.

§ 305.—Superficial Vegetations and their Attendant Phenomena.

All *Mycoderma* species share the property of forming superficial vegetations on the nutrient liquids to which they gain access; beer, wine, beer wort, grape and fruit juice, fruit wine, the residues from the distillation of beer, wine, &c. This circumstance at once gives rise to the question, Why should this peculiarity be general among *Mycoderma* species, but only shared—and in less decided degree—by a few races of the morphologically similar wine yeasts?

The explanation accepted at one time was that *Mycoderma* species require oxygen, and therefore form superficial vegetations; but this theory is imperfect. Lindner (XXXI.) and Will (XIII.) are of opinion that the cells of *Mycoderma* repel water (*see* p. 291, vol. ii.), and enclose air in their intercellular spaces, or attract air; and that it is probably this property that enables them to remain so easily on the surface. The researches of Meissner (XI.) in this direction show conclusively that air alone is the support of the film vegetations, which are themselves specifically heavier than grape juice, for instance. This air is firmly retained in the bud aggregations, which often contain many hundred cells, and are extensively branched into brush-shaped masses. In the case of wine yeasts, on the contrary, the bud aggregations, previous to the commencement of fermen-

tation, consist of comparatively few cells. It should also be remembered that, during alcoholic fermentation, in the words of WORTMANN (XV.), "the small yeast cells are whirled about in a giddy dance, and prematurely torn apart, by the ascending tiny bubbles of carbon dioxide that are soon liberated extensively, with effervescence, by the yeast itself." The bud aggregations of *Mycoderma*, on the other hand, are able to develop in quiescent liquids—hence their larger number of cells.

When a cell of any species of *Mycoderma* is sown in grape juice, beer or wine, it appears from the exhaustive researches of MEISSNER (XI.) that the phenomena of film-formation proceed in the same way as with many of the film-forming *Torulaceæ*. In a very short time the surface of the liquid is found to

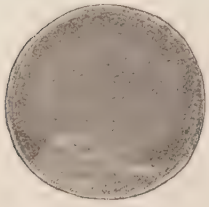


FIG. 196.—Mycoderma from Eltville red wine. First stage of film formation. Slightly reduced.

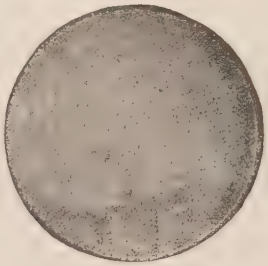


FIG. 197. — Mycoderma from Guben cider. First stage of film formation. Slightly reduced.

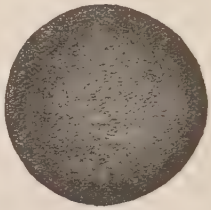


FIG. 198.—Mycoderma from Berlin white beer. First stage of film formation. Slightly reduced.

carry a vegetative growth, formed either by the coalescence of originally separate islands, or by progressive growth from the walls of the vessel. In the first stages of development, the film growth is delicate, flat and very elastic. This dull or partly lustrous surface exhibits a varying number of white spots of different sizes, more or less clearly visible, distributed irregularly or in curved lines, and representing accumulations or cells retaining air between their aggregations of buds. A peculiarity of many species of *Mycoderma*, to which attention has been drawn by LINDNER (XXXI.) and WILL (XIII.), is that the films produced by these fungi are often perforated in the earlier stages, these open spaces closing in, however, during the subsequent growth of the cells. This phenomenon still lacks explanation.

In consequence of the progressive growth of the cells, the smooth, colourless film, as in the case of many film-forming *Torulaceæ*, becomes veined, folded and wrinkled, this taking place sooner with some races than others. The initial folding differs with the various races. According to the researches of Meissner, the following four groups are well defined: the first group exhibits broad veins, protruding upward like bubbles (Fig. 196). In the second and third groups the width of the veins decreases (Figs. 197 and 198). The fourth group exhibits similar veins, but finer and of a decorative character. Under certain conditions, more especially when isolated portions of the liquid are left uncovered either in the mass of the film or against the edge of the culture vessel, the veining of the film may be preceded by the formation of parallel folds. As the film continues to grow, the veining passes over into the mesenteric form or becomes linear. In the former case the mass assumes the appearance of a loosely woven fabric, the threads of which are all entangled. These threads may be either coarse or fine. In the linear form the lines proceed either from an eccentric point in the film, or from a point on the glass wall (up which, in all cases of *Mycoderma*, the cells

climb a short distance), or again from several central points on the film or from one of the open spaces thereon. The lines running in one direction may also be uniformly distributed on the film. During the further progress of growth, alterations take place in the folding, cultures which were mesenteric at first exhibiting wrinkles of various depths. According to MEISSNER (XI.), there are five different types of corrugation: No. 1, resembling cauliflower; No. 2 having shallower corrugations; No. 3 more uniform and finer; No. 4 still finer; No. 5 showing very fine wrinkles.

The colour of the film also varies with the stage of growth and with the race. While the film is still quite thin, it has no particular colour, only a few spots (as already mentioned) showing up white. These are the places where the film has already become somewhat thicker and contains a good deal of air enclosed between the individual cells. In proportion as folding (*i.e.*, cell growth) progresses, the colour turns white at first, owing to the inclusion of air; and when the film has thickened, it becomes whitish grey, whitish yellow, whitish violet-green, violet, yellow, yellowish red, and so on. In some species a very thick film is produced, in others it remains thin, the rugous films being usually the thickest, and the more finely wrinkled forms correspondingly thinner.

With regard to the attendant phenomena in connection with the production of films, when the latter are formed on a nutrient liquid the latter may either remain limpid or become cloudy after a time, or else cloudiness may set in as the film is being formed. The cloudiness is due to the *Mycoderma* species concerned forming loose bud aggregations, the cells of which are readily detached and dispersed throughout the liquid, and the degree of cloudiness depends on the extent to which this dissemination can take place. In the case of certain species of *Mycoderma*, the detaching of the cells does not occur for some little time; and here, again, we meet with two well-defined types: either (1) large masses, or (2) small aggregations of buds, being detached from the film. On reaching the bottom of the culture vessel, the *Mycoderma* cells do not perish immediately; in fact, they are very tenacious of life, and put forth fresh bud cells from time to time. WORTMANN (XVII.) succeeded in isolating living *Mycoderma* cells from wine that had lain in bottle for 25–33 years, tightly closed with the original corks.

The starving cells at the bottom of the culture vessel may also be carried up again into the liquid by the gas bubbles rising from time to time from the deposit. These cells, being in an emaciated condition, are but little heavier, specifically, than the surrounding liquid, so that the latter occasionally remains cloudy for some considerable time.

When *Mycoderma* species are grown on grape juice or wort, the liquid may be decolorised thereby (*see* p. 294, vol. ii.). This was observed by WILL (XIII.) in the case of beer wort, and confirmed by MEISSNER (XI.) in the case of grape juice. The latter, however, observed that the pale yellow colour of grape juice may turn to a dark brown when this juice serves as a habitat for certain races of *Mycoderma*, owing to the formation of alkaline substances which neutralise the acids in the juice and finally render the liquid alkaline.

§ 306.—The Destruction and Production of Acid in Nutrient Liquids by Mycoderma.

This matter has been thoroughly investigated by MEISSNER (XI.), who obtained the following important results: The previous researches of other workers, such as KOCH (V.), WORTMANN (XV.), and WILL (XIII.), on the physiological behaviour of *Mycoderma* revealed the fact that these fungi do not invariably lessen the acidity of grape juice, wine, beer, &c.; but that some races

in particular produce acid, often in considerable quantity. Meissner (XI.) explains this phenomenon by stating that *Mycoderma* are able to form acids, as well as to destroy them, both processes going on concurrently. The preponderance of the formative or destructive action depends both on the powers of the various races and on external conditions, such as the amount of oxygen admitted to the cultures, the quantity of nutrient solution present, &c. If the acid forming capacity prevails, the total effect is an increased acidity of the medium, and *vice versâ ;* whilst if the two powers be equal, the quantity of acid remains unaltered.

In order to obtain a satisfactory insight into the nature of the process whereby a reduction of acidity is effected in liquids inhabited by *Mycoderma*, Meissner (VI.) closely examined the behaviour of these fungi when grown on artificial nutrient media (containing the necessary mineral ingredients) with different organic acids as the sole source of organic matter. The effect on malic acid was insignificant in certain races, but very strong in others. One species, isolated from Colmar wine, consumed within thirty-five days 5.72 grms. per litre (*i.e.*, 73 per cent.) of the malic acid originally present in the artificial medium, the growth of the organism increasing in luxuriance with the extent of decomposition attained. Tartaric acid proves generally ill adapted for the building up of *Mycoderma* cells, and is therefore consumed to only a small extent by the fungus. The same results were obtained by Seifert (II.). The case is different with lactic acid, Meissner (VI.) finding that six out of nine stocks examined consumed this acid extensively, the other three to only a small degree corresponding with their scanty growth. One race from Silesian perry reduced the lactic acid content of the solution (originally 0.7633 per cent.) to 0.0673 per cent. Citric acid and succinic acid were also attacked, sometimes extensively. In the former case, the acid was almost entirely consumed in one-third of the tests performed, whilst with the other acid, a similar result was obtained in one-fourth of the tests: *see* Meissner (VII.). In one-half of the same worker's experiments (VII.), acetic acid was vigorously attacked, but only slightly in three instances, whilst in three other cases the sowings failed to develop at all. H. van Laer (VIII.) in examining one race of *Mycoderma* found that it ceased to grow in wort in presence of 1.25 per cent. of acetic acid, though vigorous growth occurred in the presence of 1 per cent., four-fifths of the acid being consumed in ten days at 30° C.

The simultaneous formation of acid by *Mycoderma* has been demonstrated by the total acidity of the medium being maintained in several of the nutrient media tried, notwithstanding the consumption of the organic acids supplied. In other instances the reduction of total acidity was slight, although energetic growth of the vegetation was observed.

The various *Mycoderma* produce different volatile acids, which can be identified by the odour of the nutrient liquids. According to Wortmann (XVII.), many mouldy wines smell strongly of rancid butter, owing to the presence of butyric acid formed by the action of the *Mycoderma* on different constituents of must and wine. As long ago as 1893, Lafar (III.) isolated a film-producing budding fungus, containing *Mycoderma cerevisiæ* in the involutions of the film, from the cask sludge of a brewery where difficulties were of frequent occurrence. The beer on which the cultures were grown exhibited an agreeable, fruity smell, and, after turning sour, a pleasant taste recalling that of wine vinegar. Will (XIII.) also noticed extensive production of acid in the pale Munich beer on which his *Mycoderma* cultures were grown. "On opening the culture vessel (he says) one noticed a sour smell, at first similar to that of acetic acid, but afterwards more difficult to define, being something like baked apples. The beer had a decidedly sour taste; but not of acetic acid." It should be mentioned that

Will (XIII.) cites the authority of Raymond and Kruis for the statement that formic acid and acetic acid were discovered in a culture of *Mycoderma* that had been standing for a year at 20° C.

In addition to volatile acids, the *Mycoderma* produce fixed acids and esters, as is shown by the fact that the volatile acids formed are insufficient in quantity to account for the difference in the total acidity when an increase occurs in the acidity of the nutrient liquid. Graf (II.) mentions that the *Mycoderma cervisiæ* examined by him causes increased acidity in sterilised wort, the acid content rising in twenty-eight days from 5.7 c.c. to 8.5 c.c. in terms of decinormal baryta water. The Egyptian beverage "Leben," of the kephir type, contains a *Mycoderma lebenis*, discovered by Rist and Khoury (I.), that produces fixed acids and acetic acid. Will (XIII.) assumes that the production of acid is the cause of the decoloration of top-fermentation beer when contaminated by large quantities of the *Mycoderma* species examined by him.

§ 307.—Destruction and Formation of other Organic Substances by Mycoderma.

In addition to the organic acids, the alcohol in beer, wine and fruit wines is subjected to complete destruction by *Mycoderma* fungi, being, on the one hand, oxidised to carbon dioxide and water, and on the other employed by the fungi as an organic structural material. This last circumstance was deduced by A. Schulz (I.), who expressed the view that "the film fungus is capable of itself forming its constituent organic compounds, and requires only ammonia and alcohol for that purpose." This opinion was confirmed by Meissner (XI.). Schulz employed in his experiments an artificial nutrient solution, containing magnesium sulphate and alcohol, in addition to potassium phosphate and lime. In one case ammonium nitrate was added to the solution, as the source of nitrogen, asparagin being used in another, and ammonium tartrate in the third. The fungus thrived in all three cases, and consumed a large portion of the alcohol, thus indicating the capacity of *Mycoderma* to supply their nitrogen requirements from ammonium nitrate and to utilise alcohol in the building up of their cell contents. It must, however, be mentioned that Schulz did not work with pure cultures, and that the race used by him apparently belonged to the genus *Pichia*. An important complement of these experiments was afforded by the investigations of Meissner, as also by the previous experiments of Winogradsky (XI.), which were afterwards confirmed by A. Kossowicz (III.)—*see* p. 153, vol. ii. These showed that pure cultures of *Mycoderma* also exert the activity first recognised by Schulz. In addition to the aforesaid nitrogenous food-stuffs, Meissner (VIII.) used ammonium phosphate and chloride, which he added to an artificial nutrient solution along with the necessary ash constituents. The energetic growth demonstrated that *Mycoderma* can be supplied in nitrogen by these substances as well, and consequently part of the alcohol in the nutrient solution is oxidised, a part being utilised in building up the cell body.

The sugars are attacked by the various *Mycoderma* species in a different manner and with varying intensity. H. van Laer (VIII.), for example, reported that dextrose is not attacked in Nägeli's nutrient solution by *Mycoderma;* whereas in yeast water it forms a better food-stuff than alcohol. Maltose and saccharose are attacked in very different degree; and in this case also it appears that the degradation depends entirely on the nature of the nutrient material. "When different sources of carbon are added simultaneously to the nutrient solution, the one that is most readily assimilable is degraded first; and it is not until the alcohol has disappeared that the disaccharides are attacked." When maltose, saccharose and dextrose are added to the yeast water, the last-named sugar is

attacked first, the disaccharides not being degraded at the start. No invertase or maltase could be detected in the *Mycoderma* cells. The saccharose and maltose were oxidised direct to water and carbon dioxide. Finally, Meissner's *Mycoderma* species, which were grown on sterile grape juice, partly oxidised dextrose and lævulose, but also formed acids from these sugars. On artificial nutrient solutions, containing dextrose or saccharose as the sole organic substance, in addition to the requisite mineral food-stuffs, the *Mycoderma* oxidised the sugars, though utilising a portion of the same in the formation of new cells, and also forming acids therefrom. *Mycoderma lebenis*, Rist and Khoury (I.), grew admirably well in glucose and maltose, the glucose being transformed into acid and the alcohol oxidised.

According to further researches by Meissner (VII.), glycerine and tannin are also consumed by *Mycoderma* in the same way as alcohol, acids and sugars. A species of *Mycoderma* found by Eitner (I.) on mimosa bark also decomposed tannin.

Mycoderma species, however, in addition to decomposing glycerine, are also capable of forming the same from other organic substances. W. Seifert (II.) reports that his *Mycoderma vini I.* produced 0.152 per cent. of glycerine in Pasteur's nutrient solution by the end of 14 weeks, the whole of the alcohol having disappeared. *Mycoderma vini II.*, on the other hand, formed only 0.016 per cent. of glycerine, the alcohol diminishing concurrently from 4.8 to 4.1 per cent. by volume.

§ 308.—Influence of other Factors on the Vitality of Mycoderma.

Highly interesting observations of the longevity of *Mycoderma* in wort cultures and in the dry state were made by Will (XIII.). Cultures that had been stored for $4\frac{1}{2}$ years in wort were found to contain living cells when reinoculated in fresh wort, thus demonstrating the longevity of *Mycoderma* cells in wort. Will also showed that *Mycoderma* can survive for a long time in a dry state—at least two years in the case investigated by him. Low temperature favours the maintenance of vitality in the dry state, and the content of water in the dried cells probably also plays a principal part.

Will (XIII.) also investigated the power of old and young *Mycoderma* cells to resist the action of heat in liquids, the heating being applied in water, then in wort, and finally in sauerkraut water. The cultures were of various ages. The duration of heating was half an hour, not reckoning the preliminary warming. The results showed definitely that the degree of resistance offered by the cells is influenced by the character of the substratum. For the species under examination, the critical temperature in the heating in water test was found to be 50° C., whereas after heating for half an hour at 55° C., *Mycoderma* films were developed in all the check inoculations heated in wort. Older cultures proved better able to stand the heat than younger ones, the difference being 5° C. The formation of resting cells is not considered to account for this, Will's explanation being that the older and more strongly developed cells possess greater powers of resistance than such as are younger and more delicate. Seifert gives 0°–40° C. as the limits of temperature between which *Mycoderma* cells are capable of development, the presence of alcohol narrowing the range—for instance, down to between 2° and 33° C. in wine containing 8 per cent. of alcohol by volume. A continuous exposure of five minutes to a temperature of 60° C. is sufficient to destroy the vitality of *Mycoderma* in wine.

With regard to the influence of chemical agencies on the life of *Mycoderma* cells, the investigations of Holm and Jörgensen show that the development of the cells is accelerated by the addition of small quantities of fluorides. Siebel

found that neither yeast, *Mycoderma* nor bacteria will develop in beer that has been treated with a solution of formalin (40 per cent. solution of formaldehyde) in the proportion of 1 : 10,000; whilst in a solution of 1 : 50,000, yeast and *Mycoderma* were able to grow, but not bacteria. According to Seifert (II.), the various *Mycoderma* differ considerably in their power of resisting the influence of alcohol, development ceasing in presence of 13 per cent. of that substance by volume. In the case of furfurol, 0.5 per cent. was fatal to Will's *Mycoderma*. Sulphur dioxide is also known to be very poisonous to *Mycoderma*, and is therefore used in curing beverages that have been attacked by these organisms. Wesenberg (I.) investigated the action of antigermin, mikrosol, afral, mycelicide and antiformin on *Mycoderma cerevisiæ*, to ascertain the amount of a fatal dose. On immersion in a 2 per cent. solution of the antiseptic, the cultures, 4 days old, were killed by antiformin in ¼ hour, by antigermin in 1 hour, by mikrosol in 8 hours, and by mycelicide in 9 days, whilst afral merely retarded their growth. In a 1 per cent. solution of the poison the *Mycoderma* perished in ¼ hour with antiformin, 5 hours with antigermin, and 8 hours with mikrosol. The results were very different, however, when the antiseptics were added in definite quantity to beer wort, the growth of *Mycoderma* being then arrested by the following degrees of concentration: antigermin, 1 : 1000; mikrosol, 1 : 5000; antiformin, 1 : 20. The most powerful antiseptic, as regards the restriction of development, was undoubtedly antigermin, which, according to Wesenberg, is from 3 to 10 times as strong as mikrosol.

CHAPTER LXI.

SACCHAROMYCES APICULATUS.

By Prof. Dr. H. MÜLLER-THURGAU,

Director of the Swiss Experimental Institute for Fruit Wine and Horticulture, at Wädenswill, near Zurich.

§ 309. History, Distribution and Morphology.

Ripe, soft fruit is often found to be infested with a budding fungus, to which the name of *Saccharomyces apiculatus* has been given on account of its tapered ends. This is the fungus described by Kützing under the name *Cryptococcus vini;* and we are indebted to Reess (I). for its closer examination and for the introduction of its present name into the literature. This worker found it, associated with various *Saccharomycetes*, in fermenting fruit juices and wine musts, but never succeeded in inducing it to form ascospores. The reason why Reess in this instance left out of consideration the characteristic on which he founded the genus *Saccharomyces*, namely, the production of endospores (*see* p. 202, vol. ii.), and nevertheless applied the generic name *Saccharomyces* to this species, was on account of "its known morphological peculiarities and its physiological behaviour as an alcoholic ferment," as also "in the expectation that its power of producing ascospores will be revealed by some other method of cultivation." It was not until quite recently, however, that such a method was discovered. Hansen (IX.) tried to find it in vain, as did also Klöcker (IV.) with reference to the expression of an adverse opinion by Beijerinck (XVIII.); and consequently the so-called *Saccharomyces apiculatus* had perforce to be excluded from the family of the *Saccharomycetes* for the time being. The only reason for retaining the name bestowed upon it by Reess was a disinclination to rechristen a well-known organism.

Engel (II.) claimed to have discovered an entirely new form of fructification of this fungus, analogous to that of *Protomyces* (*see* p. 79, vol. ii.), for which reason he conferred on it the new generic name, *Carpozyma*. No one else, however, not even E. C. Hansen (IX.)—who repeated Engel's experiment—has been able to observe the form of fructification in question. The systematic position of the fungus therefore remained undetermined; and on this account the organism was separated from the rest of the *Saccharomycetes* in arranging the material for the present work.

Since that time, however, P. Lindner (XXXVIII.) has succeeded in obtaining monosporous cells in beer-wort cultures of an *Apiculatus* yeast isolated from Robinia blossoms. The drawing illustrating these cells is not very convincing, especially when it is remembered that, under certain conditions of environment, large, isolated fat globules, that can readily be mistaken for spores, are often formed in *Apiculatus* yeast. Even Reess (I.) depicted *Apiculatus* cells, each containing a round, highly refractive body resembling a spore, and expressly referred to the same as possible sporulation. Lindner's statement that only one spore occurs in each cell conflicts with an earlier communication by

Beijerinck, according to whom the *Apiculatus* cells swell up to asci containing 4–6 ascospores in each. Neither Beijerinck nor Lindner succeeded in prevailing on the "spores" to germinate. The last-named himself mentions this deficiency, and points out that some such preparation is necessary to ensure the germination of *Apiculatus* yeast as is the case with the seeds of the carob-tree, which have first to be passed through the alimentary canal of some animal. Bearing this idea in mind, A. Röhling (I.) cultivated vigorous *Apiculatus* yeast for twenty-four hours in sterilised grape juice and then used it for gypsum-block cultures, in which a "body resembling a spore" made its appearance in many of the cells by the tenth day (temperature not stated). The germination of a spore was thereafter observed in a decoction of horse-dung, mixed with 5 per cent. of grape

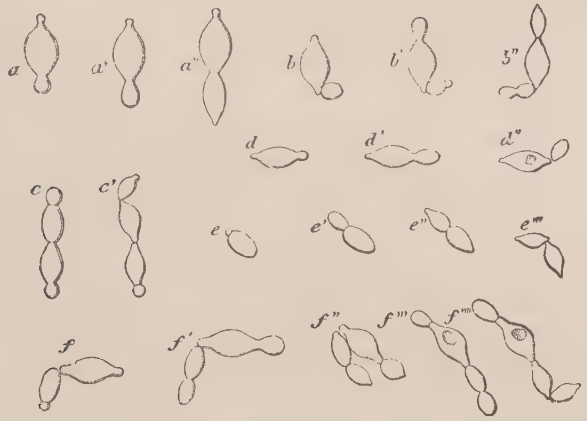


Fig. 199.—Saccharomyces apiculatus.

Typical Form. Reproduction of the cells by budding. Magn. about 950.
(*After Hansen.*)

sugar. This experiment needs repetition, from the circumstance that only one cell gave the said result, and also on account of the manner in which germination is said to have proceeded. The writer has tested four different *Apiculatus* races exactly in the same way as described by Röhling, but failed to obtain spores.

Lindner and Röhling, on the basis of their researches, concluded that *Apiculatus* yeast does sporulate, and therefore really belongs to the genus *Saccharomyces* of Reess and Hansen, but as it constitutes a peculiar type, Lindner (XXXII.) considers that a new genus should be established, for which he proposes the name *Hansenia* (*see* p. 209, vol. ii.).

The specific name, *apiculatus*, well expresses the characteristic that distinguishes this budding fungus from all others. The (otherwise ovoid) cells are pointed at both ends like a lemon (*see* Fig. 199), which form, however, predominates only during the first stage of development in a nutrient solution, whereas later on, when the conditions of nutriment are less favourable, a considerably larger number of ovoid cells make their appearance, and the lemon shape is less noticeable.

As already stated, reproduction in this fungus is effected entirely (or mainly, if sporulation indeed takes place) by budding. The progress of this operation was described by Reess and Engel, and it was more closely investigated by Hansen, whose observations show that the budding of a lemon-shaped cell proceeds in the following manner (*see* Fig. 199). The lower pointed extremity (*a*)

of the cell swells up (*a'*) and grows there until it attains normal dimensions (*a''*) The two cells then separate, each of them acquiring the hitherto lacking second tip. From *b-b''* in the Fig. it will be seen that a bud can be put forth at each end simultaneously. Figs. *c-c'* represent in each case an aggregation of four lemon-shaped cells. The question whether a lemon-shaped cell can be produced from an ovoid one by budding may be answered in the affirmative. In this case the parent cell (*e*) puts forth a bud at the one extremity, whilst the other becomes pointed (*e'*), the daughter cell rapidly increases in size (*e''*), then separates, and, together with the parent cell, acquires a point at the second extremity. R. Meissner (IX.) regards the oval shape as the normal form, and the apices of the pointed cells as incipient buds. This, however, can hardly be

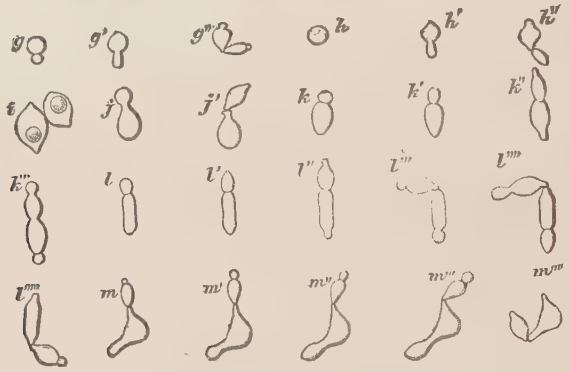


Fig. 200.—Saccharomyces apiculatus.
Abnormal cell forms. Magn. about 950. (*After Hansen.*)

accepted, since it evolves the assumption that the majority of cells cease growing just when they have begun to reach the budding stage.

A peculiar shape is exhibited by the *Sacch. apiculatus*, var. *parasiticus*, Lindner, discovered by Lindner (XXXIX.), and infesting cochineal insects. In most of the cells, one end tapers out to a long point, by means of which the eggs are infected in the body of the parent insect, and propagation is ensured in the offspring.

In addition to the oval and lemon-shaped cells already described, this fungus produces sausage-shaped growths, as shown in Fig. 200; but the external conditions under which these are formed have still to be elucidated, Reess's statement that they appear towards the close of fermentation needing confirmation, since he did not work with pure cultures. No mycelial growth has yet been observed, nor do the cells collect in long aggregations, the daughter cells separating quickly from the parent cells after bending in a peculiar way—a phenomenon that was first described by Reess (I.).

As in the case of the true *Saccharomycetes*, the dimensions of the cells vary considerably, even in the same culture, some of them measuring 2 μ, whilst others are four times that length. In the majority of instances the length is 7 μ, so that the cells are far smaller than those of beer yeasts, as can be seen from Fig. 201, where a mixture of *Sacch. cerevisiæ* and *Sacch. apiculatus* is depicted at a magnification of about 950. The first-named can be easily recognised from the greater dimensions and oval shape of the cells. On the other hand, the *Apiculatus* cells exhibit a feature that is often observed in this fungus, especially when in an unfavourable environment, namely, a large vacuole,

Nothing has been published hitherto with regard to any further morphological peculiarities of the structure of the cell membrane or contents differentiating the *Apiculatus* yeasts from the other *Saccharomycetes*.

If plate cultures of a mixture of *Sacch. apiculatus* and *Sacch. ellipsoideus* be started in must gelatin, the colonies of the former usually make their appearance only after those of *Sacch. ellipsoideus* have already attained considerable size; and they remain smaller than the latter throughout. This difference is due, not to any slower rate of reproduction on the part of the first-named yeast, but chiefly to the smaller size of the individual cells, which diminish as the colony increases. The gelatin surrounding the *Apiculatus* colonies is liquefied at an early stage, and the latter seem also to excrete substances that restrict the further reproduction of the yeast cells. A similar result is observed in the development of streak cultures of *Apiculatus* yeasts, so that, for the most part, these exhibit merely a delicate, filmy appearance, at a time when the yeast streak in cultures of *Sacch. ellipsoideus* of the same age has already grown to a thick white strip. Even after a considerable time the former do not usually show any vigorous development; and this applies also to the giant colonies, which fail to exhibit any decided, special morphological characteristics, and quickly sink in the liquefied gelatin. Further particulars on this point will be found in the next paragraph.

Fig. 201.—Cells of Sacch. apiculatus and Sacch. cerevisiæ.
Magn. about 950. (*After Hansen.*)

§ 310. Racial Differences.

Sacch. apiculatus, var. *parasiticus* (*see* p. 316, vol. ii.), must be considered as a separate variety from the *Apiculatus* yeast collaborating in the fermentation of beer and wine, since it differs from these by its strictly parasitic habit, even more than by the shape of the cells. This variety cannot be grown either in fruit juices or in artificial nutrient media. Moreover, the *Apiculatus* yeasts indigenous on fruit and playing a regular part in vinous fermentation belong to different races and are not of uniform stock, a discovery for which we are indebted to K. Amthor (I.) in 1888. Owing to the absence of sporulation and to the great morphological variability of the cells, characteristics of a chemico-physiological nature have to be mainly relied upon in demonstrating the difference, chief among them being the kind and quantity of the metabolic products furnished by cultures of different origin when grown under identical conditions. Thus, Amthor was able to prove the racial divergence of two cultures of *Sacch. apiculatus*, one of which was isolated from red Heilbronn must, and the other from a white wine must from Rhenish Hesse. Whereas the former race produced 3.65 per cent. (by weight) of alcohol and 365 mgrms. of glycerine per 100 c.c. of the grape must it was employed to ferment, the other race, under the same conditions, produced only 2.58 per cent. of alcohol and 311 mgrms. of glycerine. On the other hand, it formed a larger quantity of volatile acids, namely, 127 mgrms. per 100 c.c. as compared with 103 mgrms. in the other case. Müller-Thurgau (XXIV.) tested seven different races of *Sacch. apiculatus*, isolated by him, in grape juice, as well as in pear juice and currant juice, and found that, in the first-named medium, the production of alcohol varied between 2.5 and 3.8 per cent. by weight. The same serial order was obtained, on the basis of the rapidity of fermentation, in all three culture

media; and in every instance a high proportion of volatile acids was obtained —viz., with race 8, for instance, in grape wine 93 mgrms. per 100 c.c., and in perry 123 mgrms. (calculated as acetic acid), whereas the elliptical yeast Steinberg 1 produced only 53 and 47 mgrms. respectively in the same media. Schander (II.), who afterwards compared pure cultures of twenty-four *Apiculatus* yeasts, also observed morphological differences in the cells. In some races the cells are short, thick, and of the typical lemon shape, whereas in others they are thin and elongated, the lemon shape being less noticeable. Different races are also distinguishable by the cell dimensions. Whilst old cultures of *Apiculatus* yeasts do not generally produce a film on the surface of fruit juice or grape juice, a growth of this kind, though slight, could be detected in certain races. Racial differences were also exhibited in the form of the streak cultures and giant colonies, though not to any very decided extent. More pronounced differences became apparent in the quantity and character of the sediment and in the percentage of the fermented wines, the alcohol content being 1.44 grms. per 100 c.c. in the case of the weakest race, and 4.53 mgrms. with the strongest. Another point of racial difference consisted in the consumption of acid, and still others will be referred to in the two following paragraphs.

§ 311. Conditions of Growth and Nutrition.

In liquid media, such as beer wort and fruit juices, the reproduction of *Apiculatus* yeasts proceeds rapidly in the primary stage of fermentation, in many instances more quickly than with the ferments in technical use; but this superiority soon disappears, owing to the susceptibility of *Apiculatus* yeasts to alcohol. It is most particularly noticeable on comparing the rapidity of reproduction of *Apiculatus* and *Ellipsoideus* races in very dilute must, where the amount of alcohol formed is insufficient to retard growth, or when only the initial reproduction—up to the point when 1 per cent. of alcohol has been formed—is taken into consideration in ordinary must. No precise observations of this kind, or with regard to the amount of alcohol sufficing to stop growth under various other conditions of environment, have, however, been published. An idea of the difficulties encountered in determinations of this kind may be gathered from § 268. The rapid reproduction of *Sacch. apiculatus* in comparison with low-fermentation beer yeast is shown by certain researches performed by E. Hansen (IX.); and these also show how one of these budding fungi can retard the growth of the other when present simultaneously in the same nutrient solution. Thus, for instance, three Pasteur flasks were charged with beer wort; (*a*) being sown with 22 cells of *Sacch. cerevisiæ* per unit volume, (*b*) being 'sown with the same number, along with 19 *Apiculatus* cells, and (*c*) with 20 cells of this latter only. At the end of 13 days (at 8°–10° C.) the number of cells per unit volume amounted to: (*a*) 242 of *Sacch. cerevisiæ* (the alcohol content being 6 per cent. by volume); (*b*) 240 of *Sacch. cerevisiæ* and 45 of *Sacch. apic.* (alcohol 6 per cent.); (*c*) 791 of *Sacch. apic.* (alcohol 0.5 per cent.). Hence, in the pure culture, *Sacch. apiculatus* reproduced more than three times as much as *Sacch. cerevisiæ*, but was greatly retarded by the latter in the mixed culture. These figures of course do not afford any criterion of the rapidity of growth, since, for a given increase of growth, more than three *Apiculatus* cells must, as a rule, be formed for each one of the far larger cells of the beer yeast. This harmonises also with the circumstance that, in spite of the larger number of cells, the sediment in the fruit juices fermented by *Sacch. apiculatus* is much smaller, in weight and volume, than that in the juices fermented by *Sacch. ellipsoideus*. Consequently, in order to decide whether the protoplasm of *Apiculatus* yeast has a lower fermentative energy than that of *Sacch. cerevisiæ* or *Sacch.*

ellipsoideus, the activity per unit weight of the yeasts must be compared, and not that of an equal number of cells.

The influence of alcohol on the growth of several *Apiculatus* races was investigated by RÖHLING (I.). In harmony with the general low powers of resistance of these yeasts against injurious influences, a small percentage of alcohol in the culture liquid is sufficient to restrict reproduction considerably. When the number of yeast cells per unit volume of grape must, previous to fermentation, was = 1, it had increased to 514 in one of the races at the close of fermentation without any addition of alcohol; but, when the must contained 2.86 per cent. of alcohol, by volume, at the outset, the final number was only 192, and when the initial addition of alcohol was 4.62 per cent., only 88 cells were obtained.

The particularly frequent occurrence of *Apiculatus* yeast on berries might lead to the supposition that the juice of these fruits is specially suitable for the growth of the fungus; but comparative experiments have shown that such is not the case. According to MÜLLER-THURGAU (II.), it matters little to the development of *Sacch. apiculatus* whether malic acid or tartaric acid preponderates in the fruit juice.

On the other hand, the growth of this yeast is stimulated by the admission of free oxygen (*see* p. 184, vol. ii.). This is apparent from the behaviour of stab cultures in must gelatin, where vigorous reproduction of the yeast cells is found to be confined to the upper portion of the stab. More accurate information is afforded by an experiment of RÖHLING'S (I.), in which five different races of *Apiculatus* yeast were sown in grape juice, one portion of the latter being aerated in the fermentation vessels, the other not. A difference in favour of the aerated yeast was already apparent on the second day; and at the close of fermentation the number of cells in the aerated samples had increased from 3.3 to 9.3 times as great (according to the race) as those in the unaerated samples. This appears to harmonise with the observation that the percentage of *Apiculatus* cells is occasionally much higher in the superficial strata, and especially the head, of red wine must fermenting in open vessels than it is in the lower layers that are poor in oxygen. This need of oxygen is, of course, satisfied when the fungus is grown on the surface of solid nutrient substrata; and it is not impossible that *Sacch. apiculatus* should gain the upper hand in consequence of these specially favourable conditions. Considerable accumulations of *Apiculatus* yeast, practically in the state of pure cultures, are not infrequently found in the wounds of burst or gnawed grapes, or in the tunnels eaten out in core fruit by apple-roller larvæ.

The special conditions of nutrition of *Apiculatus* yeasts have not been closely investigated to any extent. Its requirements in respect of carbon are chiefly fulfilled at the expense of the hexoses in the nutrient medium, but the fungus is incapable of fermenting disaccharides, such as saccharose, or of utilising them for structural purposes. More on this point will be found in the following paragraph. The circumstance that the non-volatile organic acids are consumed to a larger extent in cultures of *Apiculatus* yeast in fruit juices than is the case with *Sacch. ellipsoideus*, for example, induces the idea that these acids may also be utilised as a source of carbon for building up the cells; but, on the other hand, it is equally possible that the tartaric and malic acids in the instances observed may have been decomposed by fermentation rather than used for the purpose in question. In respect of nitrogen assimilation, *Apiculatus* yeasts do not seem to differ from others; at least no statements have been published on this point.

Sacch. apiculatus appears to be more susceptible to injurious influences than the beery yeasts and the races of *Sacch. ellipsoideus* occurring in wines. Its high susceptibility toward alcohol has already been mentioned (p. 318, vol. ii.); but

though a small percentage of alcohol is sufficient to retard growth, and apparently a small additional quantity will arrest fermentation, a considerably higher alcohol content would seem to be necessary in order to kill the cells. In this connection, however, considerable differences are exhibited by the various races. Apart from this, *Sacch. apiculatus* is much more sensitive than *Sacch. ellipsoideus*, the cells of the former being generally found dead in the deposit from fermented wine. The fact that individual cells—probably of a more vigorous race—occasionally exhibit considerable longevity, was proved by a discovery of R. Braun (II.), who found living cells of *Sacch. apiculatus* in beer containing about 8 per cent. of alcohol after at least five years. Müller-Thurgau (XXIV.) also found, in his attempts to discover suitable methods of obtaining a purer fermentation of fruit and grape wines, that *Apiculatus* yeasts are killed by a quantity of sulphur dioxide (in the must) that is innocuous to the elliptical wine yeasts. Both Hansen (LVII.) and Kayser (III.) mention *Sacch. apiculatus* as particularly susceptible to the influence of desiccation; though, on the other hand, according to Will (XXXIV.) the various races differ in this respect. A. Berlese (II.) regards the fungus as offering a powerful resistance to the action of direct sunlight. The resistance of *Apiculatus* yeast to high temperatures was examined by Müller-Thurgau (XXV.), who found, moreover, that the various races differ in this particular. One of them proved to be far more susceptible to this influence than the others under examination, being killed by an exposure of ten minutes to 50° C. in grape juice, whereas the others were able to withstand ten minutes at 55° C., and thus closely approximated to the elliptical wine yeasts in this respect. The point whether the growth of the races of *Sacch. apiculatus* is dependent on temperature in a different manner from that of the true wine yeasts has not yet been investigated, though the matter is one of some importance to the conduct of the fermentation (*see also* p. 187, vol. ii.).

§ 312. Fermentation Phenomena of Apiculatus Yeast.

The fermentation set up by *Saccharomyces apiculatus* is invariably of the bottom fermentation type. In fruit and grape juices it is often confined to a merely slight turbidity, owing to the low general fermentative activity of this yeast, and also—as pointed out by Reess (I.)—because the cells being detached and not aggregated, do not offer suitable points of attachment to the bubbles of carbon dioxide.

The various races exhibit a certain uniformity in point of fermentation phenomena and metabolism, though they differ considerably from the true beer and wine yeasts. In this connection, however, the first-named have not been examined very closely. The races described by Hansen set up a vigorous, though not very extensive, fermentation in a nutrient solution containing dextrose (*d*-glucose), the resulting "head" being composed of numerous fine bubbles, and not attaining the same dimensions as that thrown up by *Sacch. cerevisiæ*, for instance. The same behaviour toward dextrose is exhibited by Amthor's races, and that of L. Boutroux (V.). According to M. Cremer (I.), lævulose or *d*-fructose is also fermented by *Sacch. apiculatus;* and the same applies also to mannose or seminose. On the other hand, F. Voit (I.), E. Fischer and H. Thierfelder (I.) are unanimous in stating that a fourth hexose, *d*-galactose, is unaffected by this fungus. So far as our knowledge extends, not a single member of the disaccharide group is fermented by *Apiculatus;* a fact demonstrated by E. C. Hansen in respect of saccharose and lactose, and by this authority and Amthor (I.) in respect of maltose. According to these workers, *Sacch. apiculatus* is capable of forming only small quantities of alcohol in beer worts, namely, from the hexoses contained therein.

The fact that this budding fungus ferments hexoses, but not disaccharides, justifies the assumption that it cannot secrete inverting enzymes like invertase and maltase; and this has been confirmed, so far as invertase is concerned, by the experiments of HANSEN (IX.). On the other hand, when the disaccharides are inverted—for instance, by heating with a little acid—they come within the sphere of action of this fungus, as is also the case when the latter is associated in the nutrient liquid with a budding fungus that secretes invertase.

In order to utilise the aforesaid behaviour of *Sacch. apiculatus* toward sugars, for the purposes of the analytical chemist, K. AMTHOR (I.) proposed to employ it in cases where small quantities of dextrose have to be determined in presence of disaccharides, *e.g.*, in beer wort, a definite quantity of the previously boiled sample being inoculated with *Apiculatus* yeast. This latter consumes only the dextrose, the percentage of which can then be estimated by the amount of alcohol (or carbon dioxide) formed. A modification of this method was proposed by A. BAU (I.), who defended it in subsequent papers (II., V. and IV.) from certain objections urged against it by H. ELION (II.). Nevertheless, in view of the susceptibility of this ferment to alcohol, he was constrained to admit that the method can only be used when the dextrose content of the sample is low, and that, even in such event, one cannot be certain whether the whole has been fermented. Even with this limitation, however, the method is useless to the practical analyst, owing to the sluggish fermentation of the hexoses. This is evident from the statements of AMTHOR (II.), who inoculated sterile wort with *Sacch. apiculatus* and found the production of alcohol amounted to 0.66 per cent. by volume at the end of twenty-seven days, 0.79 per cent. after a further fifty-four days, 1.2 per cent. nine months later, and 1.5 per cent. after a further nine months, *i.e.*, twenty-one months in all. Hence, even assuming—as has not yet been proved—that all the hexose is eventually fermented, the method must be regarded as far too slow for practical purposes. The trouble bestowed on its elaboration, however, has not been wasted, since it affords physiological confirmation of the previous discovery of certain chemists, *e.g.*, H. BUNGENER and L. WEIBEL (II.), that beer worts contain a larger proportion of fermentable sugars, other than maltose, than had been hitherto supposed, amounting probably to one-fourth or one-third of the total sugar.

On the basis of experiments, which we cannot go into more fully here, E. DUBOURG (I.) formed the conclusion that yeasts can be induced, by habituation, to utilise certain sugars that they are normally incapable of attacking. A yeast, for instance, that is unable to invert saccharose may acquire this capacity by being grown in a mixture of glucose and saccharose, and then transferred to a solution of saccharose containing suitable yeast foods. Unfortunately, the yeast was not properly identified in the report. A. KLÖCKER (IV.) repeated Dubourg's experiment with a number of yeasts characterised by the peculiarity in question, among them being the one with which we are more particularly concerned at present, namely, *Sacch. apiculatus* (*see* pp. 190, 191, vol. ii.). The results, however, were entirely negative, our budding fungus being unable to ferment saccharose after this preparatory treatment and therefore—contrary to Dubourg's hypothesis—incapable of secreting invertase.

Despite the initially rapid reproduction of *Apiculatus* yeasts in grape and fruit juices, the fermentation they induce produces but little alcohol, proceeds slowly, and therefore usually extends over a considerable period of time. This is evident, for example, from the series of experiments recounted by MÜLLER-THURGAU (XIV.). At 14° C., the quantity of carbon dioxide liberated amounted, during the first ten days of fermentation in grape juice, to 6.8 grms. per litre; in 20 days, 9.4 grms.; in 40 days, 12 grms.; in 80 days, 14.6 grms.; in 100 days, 15 grms.; in 130 days, 16.8 grms., and in 205 days, 18 grms.; consequently

the *Apiculatus* races are all weak ferments, though differing considerably in relative degree. Under ordinary conditions of fermentation, the quantity of alcohol finally produced (*see* pp. 317, 318, vol. ii.) by the races hitherto described varies between 2.5 and 4.5 per cent. by weight; though two of the races recently tested by the writer formed 6 per cent. of alcohol from grape juice. The final content of alcohol also differs correspondingly in pear juice and grape juice, even when the same yeast is employed in both cases, Müller-Thurgau having found that *Apiculatus* race 8, for instance, furnishes 2.8 per cent. of alcohol in grape juice, as compared with 3.5 per cent. in pear juice. It may be assumed that with this budding fungus, as with others, the activity as well as the growth of the individual cells will be influenced by the conditions of nutrition and general environment, the relative speed of fermentation and the attenuation obtained with liquids of different constitution being affected both by the number of active yeast cells and the fermentative activity of the individual cells. It is, however, not impossible that, in certain circumstances, only one or the other of these two factors will come into play; but at present no systematic investigations have been carried out to decide this question.

It has already been mentioned (p. 319, vol. ii.) that the reproduction of *Apiculatus* yeasts is stimulated by the admission of free oxygen or air; and all that now remains is to deal with the influence of the oxygen supply on the fermentation process. Röhling (I.), who experimented with several races, found them to be powerfully stimulated by the admission of oxygen, and enabled to produce much larger quantities of alcohol in grape juice. In the absence of a supply of oxygen the final percentage of alcohol varied between 2.27 and 3.03 per cent. by weight, rising to between 5.01 and 5.76 per cent. when oxygen was supplied. Since only 0.2 per cent. of unfermented sugar remained in the sample furnishing the maximum quantity of alcohol, the yeast in question would probably have been able to produce still more alcohol in a stronger juice. These experiments show that the production of alcohol was doubled on the average by the provision of a supply of oxygen, the vital energy and power of resisting alcohol being considerably increased, to a greater extent than had hitherto been observed with elliptical wine yeasts. When oxygen is supplied, the fermentation proceeds more rapidly from the start, and, despite the higher final content of alcohol, is terminated sooner than in the check experiment without oxygen.

Certain chemical substances met with in vinous fermentation, such as acetic acid, sulphur dioxide, and tannin (*see* pp. 179–181, vol. ii.), restrict the growth of *Apiculatus* yeasts, and also probably exert a direct lowering influence on the fermentative energy, in the same way as they do in respect of beer yeasts and wine yeasts. According to the experimental results communicated by Röhling (I.), even 0.1 per cent. of acetic acid exerts a decided influence on fermentation; 0.5 per cent. restricts the fermentation to about one-third the normal, and 1 per cent. practically arrests it altogether. In the case of sulphur dioxide, as little as 0.025 per cent. suffices to stop the fermentative activity of *Sacch. apiculatus* almost completely; and, in leed, the earlier discoveries of Müller-Thurgau (XXIV.) show that even 65 mgrms. of this substance per litre, *i.e.*, 0.0065 per cent., will bring about the same result. Tannin is less energetic, no considerable restriction of fermentation being observed until the tannin content reaches 0.5 per cent.

During the fermentation of fruit and grape juices by *Apiculatus* yeasts, the non-volatile or fixed organic acids, *i.e.*, tartaric acid and malic acid, are also involved in the process of metabolism (*see* p. 150, vol. ii.). This was demonstrated by Müller-Thurgau (XIV.), in whose experiments the races under investigation reduced the amount of non-volatile acids in grape juice from 0.883 to 0.669 per cent.—that is to say, by about 24 per cent. of the original quantity—and in pear

juice from 0.450 to 0.265 per cent., a reduction of about 40 per cent. (In the latter case, also, more alcohol was produced.) Elliptical yeasts, employed with the same juices for the sake of comparison, consumed a smaller proportion of fixed acids; and the greater activity of *Sacch. apiculatus* toward these acids was also apparent in mixed cultures. Nevertheless, since various acids are present in fruit and grape juices, and acids (*e.g.*, succinic acid) are also formed during fermentation, these experiments, though of great technical interest, are incapable of affording a complete solution of the behaviour of *Apiculatus* yeasts toward acids. This is more likely to be obtained by fermentation experiments with liquids containing only a single organic acid, and of simple, known chemical constitution. Schukow (II.) showed, in a single experiment, that *Apiculatus* consumed a larger quantity of acid than the beer and wine yeasts, when grown in an artificial nutrient solution containing both tartaric acid and malic acid. Additional researches in the same direction would furnish valuable results. Of late the behaviour of various fungi toward lactic acid has been investigated by Meissner (X.), *Sacch. apiculatus* being also borne in mind. These experiments, however, were performed with artificial solutions, lacking fermentable sugars, so that, possibly, the behaviour of the organisms would be different from that in fermenting liquids more favourable to development. Whereas, in the solution containing mineral substances, peptone and lactic acid, various species of wine yeasts decomposed 70 per cent. and more of the lactic acid, the diminution produced by one of the *Apiculatus* yeasts—which exhibited only very slight reproduction—was only 0.018 per cent., or 1.5 per cent. of the initial quantity. Two other races proved incapable of growing at all in the solution. In fermented wine the decrease of lactic acid under the action of an *Apiculatus* yeast was less, on the average, than with the elliptical yeasts, no doubt on account of the greater restrictive influence of alcohol on the former.

The consumption of acid may also be accompanied by a production of acid (both volatile and fixed), in which the *Apiculatus* yeasts likewise play some part. Of the fixed acids, succinic acid has long been known as a fermentation product, and is also produced by *Sacch. apiculatus*. In the case of the two races examined by him, Amthor furnished definite proof that they produce considerable quantities of fixed acids during fermentation, the one forming 0.37 per cent. (calculated as tartaric acid), or three times as much as Pasteur found in fermentation with ordinary yeast. To this must also be added the amount eliminated by decomposition processes. Meissner has also shown that *Sacch. apiculatus* can produce lactic acid from succinic, malic, and citric acids, in which respect it is but little inferior to the wine yeasts. Nevertheless, since the acidity of the liquid is diminished, rather than increased, by the conversion of the aforesaid organic acids into lactic acid, this cannot explain the considerable increase of the total fixed acids reported by Amthor. Unless one is disposed to assume that the newly formed acid is succinic acid exclusively, it must be concluded that fixed acids of some other kind are also formed during fermentation by *Apiculatus* yeasts.

The well-defined power of *Sacch. apiculatus* of producing volatile acids in large quantities has already been mentioned on pp. 317, 318, vol. ii., together with the figures of production given by various workers. In the opinion of Müller-Thurgau (XI.), these volatile acids constitute a weapon by which this fungus is able to restrict the development of other yeasts. The nature of these volatile acids, however, cannot yet be precisely stated. In any case, as Amthor (I.) showed, by the preparation of the silver salt, they consist only partly of acetic acid; and moreover, according to Müller-Thurgau (XVII.), the wines do not exhibit the characteristic flavour of acetic acid. In experiments with a sterilised nutrient solution containing ammonia salts, dextrose, and invert sugar, and

inoculated with *Sacch. apiculatus*, Amthor (II.) detected in the distillate from the fermented liquid both acetic acid and formic acid, together with traces of an acid boiling at 120°–125° C. The fixed acids consisted of succinic acid and lactic acid. The first-named probably combine in part with the alcohol to form esters, which do not taste or smell sour in the liquid, but are decomposed in distillation so that the acid is available for determination. These esters, which *Sacch. apiculatus* is capable of producing to a larger extent than other yeasts, are the chief cause of the fruity flavour exhibited by the musts, worts, and fruit juices fermented by means of this fungus. According to the experiments of W. Seifert (IV.), *Sacch. apiculatus* produced a larger quantity of volatile esters (together with 0.064 per cent. of volatile acids) from one and the same grape must than was furnished by six pure-culture yeasts. The ester content, expressed in c.c. of decinormal alkali per 100 c.c. of wine, was 10.8; whilst in the case of the other yeasts it ranged between 1.32 and 4.4. P. Lindner (VII.) observed an extensive production of fruity ethers by an *Apiculatus* yeast, more particularly when the liquid under fermentation was vigorously aerated and contained a sufficiency of dextrose. In addition to the sweet-smelling esters, *Apiculatus* yeasts may also produce other kinds of odorous and flavouring substances under certain conditions. In fact, H. Will (V.) succeeded in effecting a means of differentiating the various species isolated by him, as pure cultures, from wort, beer, grapes, &c., according to the character of the smell they produce. One series is distinguished by the mouldy, fusty smell of the cultures, whilst another exhibits a very decided bouquet (fruity smell) resembling amyl ether. In saccharine yeast water they produce acetic ether, more particularly when the nutrient solution is aerated continuously. According to Schander (III.), some races of *Sacch. apiculatus* are to be numbered among the yeasts that produce sulphuretted hydrogen, and its accompanying disagreeable taste, in wine.

As another metabolic peculiarity may be mentioned Henneberg's (VI.) discovery that the cells of this yeast are only able to store up small quantities of glycogen. On the other hand, the abundant secretion of proteolytic enzymes is demonstrated by the rapid liquefaction of gelatin in cultures of various *Apiculatus* yeasts. Finally, Amthor (I.) mentions, as a special property of these budding fungi, that it exerts a powerful decolorising action when employed to ferment wine must, which observation was confirmed by Schander (II.) in the case of the races examined by him.

§ 313. The Importance of Saccharomyces apiculatus in Wine-making.

Reess (I.) found *Sacch. apiculatus* to be a constant member of the abundant fungoid flora on ripe grapes and fruit; and this discovery has since been confirmed by Pasteur (XXVIII.) and numerous other workers. Hansen (IX.), in his researches on the life cycle of yeasts, showed whence and how this yeast finds its way on to grapes, the easily recognisable cell form of *Sacch. apiculatus* being specially adapted for this purpose. The particulars have already been given in § 271. Cells of *Sacch. apiculatus* are only found occasionally on unripe fruit, on which they soon perish, owing to the unfavourable conditions, especially drought; but as the fruit ripens, the conditions of existence become more suitable for the fungus. It is first found on cherries, these ripening earlier than any other fruit (in Central Europe); soon afterwards it is met with on gooseberries and currants, then on plums, and, finally, on grapes. Strawberries, raspberries, and sorb apples come in in their proper turn. Where two kinds of fruit that ripen at different times are met with, even side by side, such as currants and grapes, only the earlier one will be found infested with the fungus at first, the

other exhibiting none at all or only a few isolated cells. Thus, in a vineyard at Geisenheim-on-Rhine, where a plantation of early Burgundy grapes lay side by side with one of late grapes of the same class, MÜLLER-THURGAU (XXVI.) observed on August 23 large numbers of yeast cells on the earlier grapes, that were just ripe, whereas none could be found on the adjoining (unripe) late grapes. Apart from the fact that the wind-borne yeast cells found a more suitable habitat on the ripe grapes, and were able to reproduce extensively thereon, especially when they could gain access to the juice through any aperture, it is certain that —as discovered by Müller-Thurgau—a considerable part is played by insects, which prefer ripe fruit, and, in visiting the latter, transfer yeasts from wounded berries to sound ripe ones, which latter they also wound in many cases, and thereby infect. It was at one time considered that the transference of budding fungi in this way was chiefly effected by the legs, maxillary organs, and the hairy parts of the insects; but A. BERLESE (II.) showed that yeasts—especially *Sacch. apiculatus*—are present in the alimentary canal of various insects, and also reproduce abundantly therein, so that the organisms are transferred to the fruit in the excrement. In fact, Berlese regards this as one of the most important means of disseminating the fungus in question.

Considerable divergence exists in respect of the numerical ratio in which the various organisms occur on the ripe fruit, and subsequently in the expressed juice therefrom; and as this greatly influences the progress of fermentation and the character of the fermented product, it is desirable that the conditions determining this ratio should be more closely examined. Thus, in view of the great influence exerted by the *Apiculatus* yeasts, it is highly desirable to trace out the factors by virtue of which the fruits used in wine-making are infested one year by a relatively large number of these fungi, whilst another year there are comparatively few; and also why the ratio between elliptical and apiculate yeasts is favourable at one time and unfavourable at another, according to the external conditions of environment. At present we know very little about this matter.

Reess, who verified the frequent occurrence of *Sacch. apiculatus* on grapes, found experimentally that in many cases this fungus assumes the duty of starting the primary fermentation of wine must, being afterwards displaced by *Sacch. ellipsoideus*, which is then in a state of vigorous growth and carries the fermentation to completion. The fungoid flora of a large number of grapes from different districts was investigated by MARTINAND and RIETSCH (II.). In one case they found *Sacch. apiculatus* exclusively on eight varieties of grapes; and in three others 20 per cent. of *Sacch. ellipsoideus*, with 80 per cent. of *Sacch. apiculatus* and *Mycoderma*. In another case they allowed crushed grapes to ferment, and found only mould fungi and *Sacch. apiculatus*; and it was only after repeated experiments that a few colonies of *Sacch. ellipsoideus* could be detected. Musts obtained from Marcobrunner grapes contained 80 per cent. of *Sacch. apiculatus*, those from Johannisberg grapes containing 25 per cent., whilst in a few others the proportion was lower. Of course it must not be forgotten that the constitution of the fungoid flora on packed grapes is liable to alteration if transmitted to a distance. MÜLLER-THURGAU (XXX.), however, has found that fresh grapes are frequently infested with very large quantities of *Apiculatus* yeasts, accompanied by only small numbers of elliptical yeasts. Thus, *Sacch. apiculatus* alone was found on grapes from Bernegg (Rhine valley) and Winterthur; and to the extent of 93 per cent. on grapes from a vineyard at Brestenberg (Aargau); though in other instances the proportions were more in favour of *Sacch. ellipsoideus*. The above figures refer to single specimens of grapes; but of course the fungoid flora may vary on grapes from adjoining stocks or even from the same vine, so that there is no instance on record of wine must being imperfectly fermented owing to the absence of any other yeasts than *Sacch. apiculatus*. Such cases, however,

have been known with currant wine; though why they should occur with this class of wine and not with grape wine is unknown. *Sacch. apiculatus* is also frequently present in abundance in the fermentation of cider and perry, at least at the outset, to a greater extent than in grape must.

The influence of *Sacch. apiculatus* in collaboration with *Sacch. ellipsoideus* (mixed sowings) in the fermentation of wine has been studied by Müller-Thurgau (XXX. and XVI.). Various wine yeasts were sown in flasks containing equal quantities of previously sterilised must, the yeasts being used alone in one series, and conjointly with *Sacch. apiculatus* in another. The number of yeast cells in each sowing was approximately the same, so that the flasks with mixed sowings contained about twice as many cells as those sown with wine yeast or *Apiculatus* yeast exclusively. The amount of carbon dioxide liberated, and consequently the progress of fermentation, was ascertained daily by weighing the flasks. The results obtained in this way with two of the yeasts during the early stages of fermentation have been plotted in Fig. 202, the abscissæ expressing the duration of fermentation in days, and the ordinates the total amount of carbon dioxide (in grms. per litre of the liquid under examination) liberated—as determined by the loss in weight—up to the corresponding days. The line marked by crosses shows the progress of fermentation with Steinberg yeast No. 1, a vigorous white wine yeast from the Rheingau, whilst the dotted line represents the work done by a weak red wine yeast from Karthaus, near Ittingen (Canton of Thurgau), and the unbroken line the progress of fermentation in the sample inoculated with *Sacch. apiculatus* (3) only. The small circles correspond to the carbon dioxide determinations. Other particulars can be gathered from the chart itself, especially the difference in the progress of fermentation with the two yeasts, and the considerable extent to which their fermentative activity was impaired by the *Apiculatus* yeast. Even so vigorous a yeast as Steinberg No. 1 was greatly retarded by the pointed yeast at the outset, so that, on the 19th day, for instance, the total disengagement of carbon dioxide amounted to 38.1 grms. with Steinberg No. 1, 6.8 grms. with *Apiculatus*, and to only 11.6 grms. in the sample with both yeasts, notwithstanding the double sowing. In proportion, however, as the percentage of alcohol increases, the *Apiculatus* yeast is weakened, and its injurious influence on the wine yeast diminished. This is clearly evident from the chart. The influence of *Apiculatus* on the weaker red wine, Karthaus yeast is still more adverse than with the Steinberg yeast, and is more clearly evident from the chart. On the 20th day the amount of carbon dioxide liberated was 39.6 grms. with the Karthaus yeast alone, 9.4 grms. with *Apiculatus*, and 10.8 grms. with the two together, so that the wine yeast had hardly come into action at all by that time. However, as soon as the proportion of alcohol reached 1 per cent. by weight, the fermentation increased rapidly, more especially from the 40th day, when the alcohol content had attained about 2 per cent., the wine yeast being then able to free itself from the *Apiculatus* enfeebled by the alcohol.

The cause of the restrictive influence of the *Apiculatus* yeasts on the fermentative activity of wine yeasts has not yet been definitely ascertained. Müller-Thurgau (XXX. and XVI.) has shown that when *Apiculatus* acts in conjunction with wine yeast in fruit must or grape must, smaller quantities of yeast are formed than when the latter is acting alone. It is, however, probable that the former restricts the fermentative activity of the individual cells, as well as their growth, either by lowering their fermentative energy or shortening their period of activity, or both.

The active agent in restricting the other yeasts probably consists chiefly of volatile acids. True, as Müller-Thurgau (XXX.) asserts, the quantity of these acids is smaller than would correspond to their powerful adverse influence, at least if they consisted entirely of acetic acid; but the more powerful formic acid

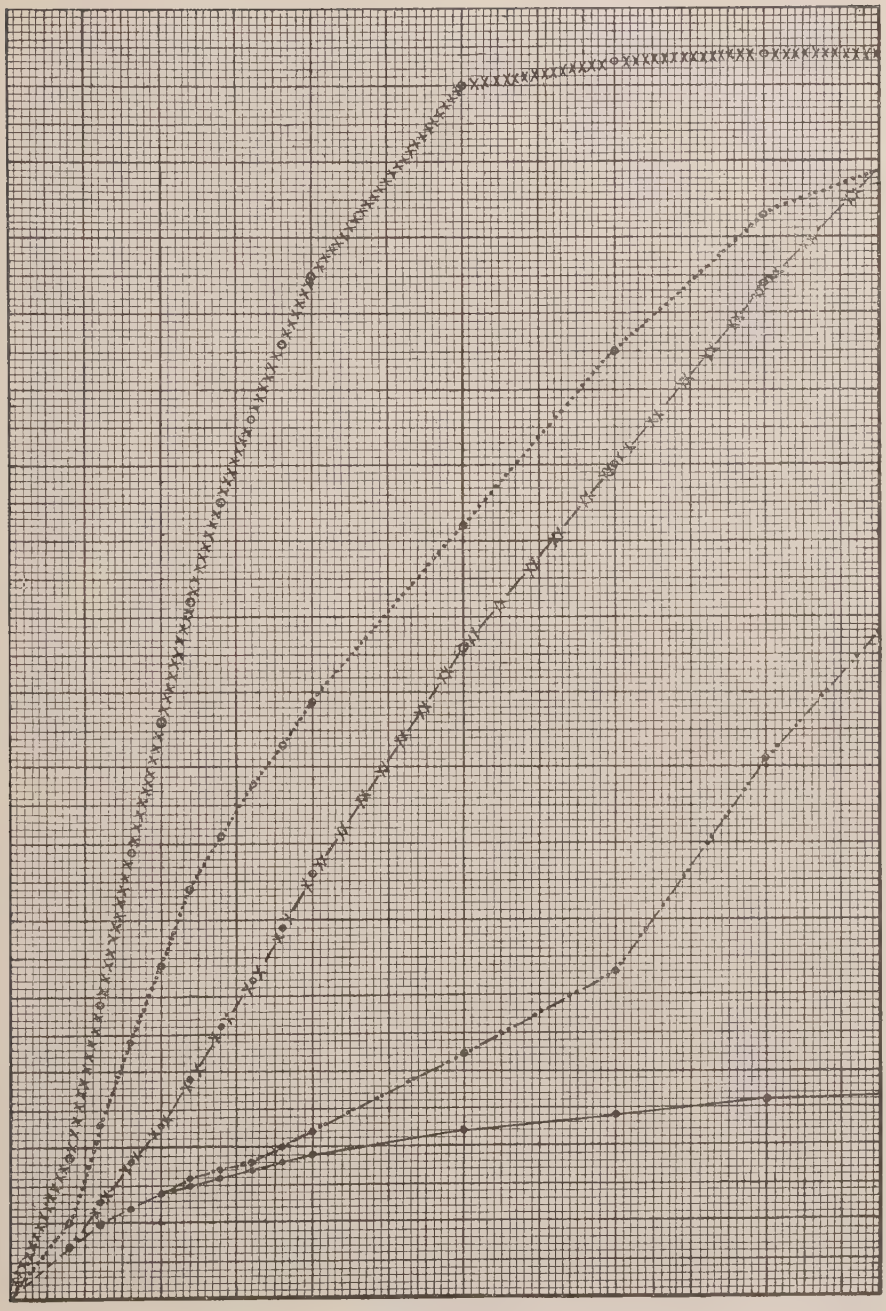


FIG. 202.—Influence of Saccharomyces apiculatus on the fermentative activity of two kinds of wine yeast:

Steinberg yeast only.
Karthaus yeast only.
S. apiculatus only.
Steinberg yeast + S. apiculatus.
Karthaus yeast + S. apiculatus

2 mm. = 1 day = 1 grm. of carbon dioxide.

is also present, and probably other substances capable of retarding growth and fermentation. When the fermentative action of *Sacch. apiculatus* is restricted by the liberated alcohol, the further production of these injurious substances ceases; and at the same time the progress of fermentation indicates that the injurious substances already present are either destroyed or converted into others of less power (*e.g.*, the volatile acids into esters).

Experiments prove that the more vigorous the elliptical yeast the less is it affected by *Apiculatus* yeast, on the one hand because of its greater power of resisting injurious influences, and also because it produces more rapidly the requisite quantity of alcohol for checking the enemy. On this account the difficult period of the struggle will be traversed more quickly when the proportion of *Sacch. ellipsoideus* to *Sacch. apiculatus* is greater in the sowing. In fact, experiments performed on this point by Röhling (I.) showed that when a large proportion of elliptical yeast is sown along with a small quantity of *Apiculatus* yeast, the latter is kept in the background and fermentation is scarcely hindered at all, though retardation occurs when even a small extra additional quantity of volatile acid is formed. The best flavoured wines are those in which the number of elliptical yeast cells in the sowing was high in comparison with those of the apiculate cells.

The collaboration of *Sacch. apiculatus* in the fermentation of wine is always a drawback, not merely because fermentation is retarded, the fixed acids destroyed in an uncontrollable manner (and one that may proceed too far in certain beverages that are low in acid), and volatile acids and other malodorous and bad-flavoured products, or such (esters, &c.) that may alter the character of the beverage, are formed; but also because in many instances the attenuation is unsatisfactory. For example, Müller-Thurgau (XVI.) has found that both fruit and grape wines fermented with an elliptical wine yeast and a race of *Apiculatus* mostly contain a large residue of unfermented sugars of the kind the latter yeast cannot attack. In the case of the experiments detailed on pp. 326–328, vol. ii., the amount of these sugars left in a grape wine at the close of primary fermentation was 10 per cent. with *Sacch. apiculatus*, 0.05 per cent. with Steinberg yeast, 0.075 per cent. with Steinberg and *Sacch. apiculatus*, 0.189 per cent. with Karthaus yeast, and 0.396 per cent. with Karthaus and *Sacch. apiculatus*. Moreover, it is well known that wines low in alcohol are more liable to maladies like turning (acetic taint), and more especially ropiness, when they also contain an appreciable residuum of sugar capable of furnishing the corresponding pathogenic organisms with material for nourishment or fermentation.

The wine and fruit wine industries are in the unpleasant position of having to make the best of the presence of *Sacch. apiculatus*. In the case of apples and pears a large proportion of the indigenous yeast can be removed by washing, but this is impracticable, for several reasons, with berries and especially with grapes. The purification of grape must by filtering or centrifugalising is attended with insuperable difficulties at present, and pasteurisation is mostly impracticable under the existing conditions of the wine industry. On the other hand, the growing employment of pure culture wine yeasts affords a suitable means for rapidly suppressing the injurious influence of the *Sacch. apiculatus* present in the indigenous yeast. Whenever it is anticipated that fruit must will be strongly infected with the pest, or the presence of the latter has been discovered in the microscopical examination of grapes or the freshly expressed juice of same, it is advisable to employ more than the usual quantity of pure yeast, and to add it to the must as early as possible. In making cider and perry the fermentation of the beverage will be purer and the content of volatile acids smaller if the fruit be washed thoroughly before putting it through the mill, and the juice be then pitched with a sufficient quantity of vigorous pure yeast. Even in these circum-

stances *Sacch. apiculatus* will have some opportunity during the must stage and at the commencement of fermentation of running riot for a short time. This may, however, be prevented, and a purer fermentation achieved by adopting the suggestion of Müller-Thurgau (XXIV.), viz., killing the more susceptible fungi (*Apiculatus* yeast included) with sulphur dioxide, and starting fermentation with a vigorous pure yeast that has, if necessary, been habituated to that reagent. Nathan (I.) claims to have prevented the development of *Apiculatus* yeast in berry juices by an initial addition of 2 per cent. of alcohol, though it stood the ordeal better when treated with 10–15 per cent. of fermented grape or berry wine immediately after pressing. This recommendation, however, does not seem to have found application.

Wines made by the pure-fermentation process are not always preferred by consumers, at least in the case of fruit wines, many liking the more strongly flavoured and odorous fruit ethers and esters generated by *Apiculatus* yeasts, especially in beverages otherwise poor in bouquet. Probably this accounts for the conflicting opinions expressed in the literature with regard to the influence of these budding fungi on the flavour of fruit wines. The fact that wines fermented with the powerful aid of *Apiculatus* yeasts give, on analysis, high volatile-acid values without being sour, forms a matter of considerable interest to the foodstuff chemist.

CHAPTER LXII.

THE MONILIÆ AND OIDIA.

By Dr. H. WICHMANN,

Deputy Manager of the Austrian Research Station
and Academy of Brewing, Vienna.

§ 314. Monilia, Sachsia and Chalara.

Among the organisms now classed as fungi imperfecti (pp. 19, 20, vol. ii.), the species assigned to *Monilia* have a particular interest for the fermentation technologist, the species in question forming a connecting-link, morphologically speaking, between the mould fungi and the budding fungi.

The members of the genus *Monilia* lack, in the first place, the complete mycelium exhibited by the mould fungi, *e.g.*, *Penicillium*; and, though divergent and branched hyphæ are by no means uncommon, the structure of the mycelium is very loose. For this reason the films produced by certain of the species when grown on liquid media are easily disintegrated, and exhibit a greater resemblance to mould films. On the other hand, the bud mycelia, the usual form of growth in this genus, show a more extensive polymorphism than is ever found among the true budding fungi; and it is this peculiarity that forms the characteristic feature of the genus. The bud mycelium, especially when aged, mostly exhibits all the forms observed in budding fungi from globular cells, resembling *Torula*, to elongated cells like those of *Mycoderma*, and even tubular cells of remarkable length, these being interspersed by cells analogous to those of *Oidium* and radial hyphæ of typical structure. These mycelia, in addition to appearing in nutrient liquids, constitute the normal form of growth on solid, moist substrata, so that, *e.g.*, the giant colonies on wort gelatin resemble yeast rather than moulds. Another regular phenomenon is that the vegetations of one and the same species on different nutrient media exhibit such a great divergence of cell form that no one would attribute them to the same species.

Another feature equally of diagnostic value is the absence of characteristic organs of fructification. In most species it is impossible to speak of fructification at all; and even in the few species, like *Monilia sitophila*, which throw up hyphæ resembling conidiophores, the difference between vegetative cells and reproduction cells is very slight and confined entirely to the shape. Thus, in *Monilia sitophila*, both the conidia and the mycelial cells are of a uniform orange-yellow colour. In some species, *e.g.*, *Monilia variabilis*, the tubular or *Oidium* cells occasionally display unevenly distributed tubercles or points, on which the yeast-like conidia are sessile, and which might be regarded as sterigmata; but this is all. Hence, in *Monilia*, we have merely to deal with vegetative yeast conidia (*see* p. 16, vol. ii.), which do not differ materially from the cells of the bud mycelium either in shape, contents or origin.

A comparison of the budding cells of a *Saccharomyces* and a *Monilia* easily reveals a remarkable difference in the appearance of the protoplasmal contents. In *Monilia* these are more delicate, homogeneous, so that the cell is lighter in

appearance, and the large vacuoles, invariably present, contain a spheroidal granule that is in constant rapid motion. According to A. Guilliermond (VI.), these bodies are identical with Babes' metachromatic granules or Butschli's red granules, and are similar to the chromatin granules in bacteria. Hansen and Guilliermond state that a cell nucleus is present; but, as in most cells, it is not visible.

Monilia candida (Bonorden), Hansen, affords the finest examples of the typical forms of growth. E. C. Hansen (XLVI.) investigated this fungus, and identified it with a species described by Bonorden (I.). The morphological variations of this species are highly diversified. When grown on sweet fruits or fresh cowdung, it appears as delicate mycelial filaments, whereas in saccharine liquids and on solid media a yeast-like growth predominates (*see* Fig. 99). This latter form is seen at its best in hopped beer wort, where the globular to ellipsoidal cells produce buds actively, so that, as in the case of top yeasts, small aggregations of cells are formed, containing characteristic elongated buds that are at once noted by the experienced observer. These cell forms are chiefly found in the sediment, whilst the quick-growing film, though initially of the same type of cells, afterwards consists of a vegetation resembling mould with greatly elongated, radial hyphæ that partly develop into numerous yeast conidia and partly disintegrate like *Oidia*. The mycelium of the film consists therefore of an intricate mixture of true hyphæ, aggregated buds, bud cells and *Oidium* cells. On solid media like wort gelatin, the colonies resemble those of yeast, with a puffy, corrugated centre and flat, fibrous rim, the growths resembling yeast cells being situated in the central portions of the colony and the mycelial forms in the outer portion. *Monilia candida* is characterised by considerable enzymatic power, and for a long time served as a typical example of a fungus capable of fermenting saccharose direct, that is to say, without the assistance of an inverting enzyme, until it was discovered to possess an endoenzyme of this class, namely, *Monilia* invertase (*see* chap. lxv.), by Emil Fischer and P. Lindner (III.), as also by E. Buchner and J. Meisenheimer (I.). The alcoholic fermentation is most active in glucose solutions, and weakest in saccharose; in beer wort the production of alcohol amounted to 1 per cent. by volume in 14 days, and to 6.7 per cent. in 26 months. Pure maltose is fermented very readily, and completely so in a yeast-water solution; and the fact that, in addition, a true dextrin is decomposed that beer yeast is unable to attack, explains A. Bau's (XXI.) discovery that beer wort can be more completely fermented by *Monilia candida* than by beer yeast, though the operation proceeds more slowly. The fermentation is accompanied by the formation of volatile by-products that restrict the process. Fermentation in grape must furnished 6 per cent. by volume of alcohol (as compared with 14 per cent. in the case of true wine yeast) in about 3 weeks, and—as reported by E. Mach and K. Portele (III.)—the resulting wine had a decided peculiarly fruity flavour. The fermentation temperature is relatively high, the maximum being about 40° C. For the vegetative processes the maximum temperature is 42°–43° C., and the minimum 6°–4° C., the fungus being therefore apparently a lover of warmth. According to Hansen, the metabolic products include acids (lactic acid ?) and nitrites, the latter having been found in barely detectable quantities by A. Maassen (I.). This species is of very widespread occurrence. W. Bräutigam (II.) found it as the chief fungus in sugar refinery waste and brewers' grains, and also in the dung of cattle fed on the first-named material. Other, morphologically similar, fungi are often classed as *Monilia candida*, even though they do not exhibit all the characteristic features of same. Thus, Adametz (XIII.) describes an example of this species from arable soil, Marpmann (VII.) reports its occurrence in cheese, and Harz (I.) in Allgau cheese and also on hay, dried plums and drum figs. According to Aderhold (V.), it is found in pickled gherkins; and Behrens (III.) observed it in the preliminary

fermentation of tobacco. It should also be mentioned that B. Fischer and Brebeck (I.) observed "endogenous cell formation" in *Monilia candida*, and wished to classify this fungus with the genus *Endo-blastoderma* (*Blastoderma*, *see* p. 300, vol. ii.) of their system: a proceeding that cannot be sustained in view of the criticisms of Lindau and Lindner.

Monilia variabilis, Lindner, is a species characterised by extensive polymerism, and was discovered by P. Lindner (XII.) on Berlin white bread, as greyish white, mealy patches, resembling *Oidium lactis*, but mostly consisting of torulaceous cells. These form heaps of larger or smaller dimensions, between long, cylindrical, almost empty cells, supporting small tubercles carrying isolated torulaceous conidia. These latter, which measure 1.8–4 μ in diameter, swell up in beer wort to as much as 8 μ and over in diameter before germinating, and usually develop a branching chain of ellipsoidal cells. The terminal cells frequently become filamentous, divide and disintegrate like *Oidium*. It is worthy of note that, in small-drop cultures, these filaments grow on the surface of the drops, and produce aerial, torulaceous conidia, which frequently mask the *Oidium* cells completely (Fig. 203). In surface cultures the torulaceous cells predominate, whereas when air is excluded, cells resembling yeast and *Dematium* are formed. Hence, in its various stages of development, this *Monilia* exhibits all the different cell forms found in the budding fungi: *Dematium* forms, *Oidium* forms, *Saccharomyces* forms and *Torula* forms; and consequently, in any given case, any cells of *Saccharomyces cerevisiæ* accidentally present could not be distinguished from the cells of *Monilia*. On beer wort, *Monilia variabilis* quickly produces a dry, loose, mealy film, which is readily disintegrated, but in time grows to a thickness of about 1 cm. and acquires considerable strength. It consists chiefly of torulaceous cells, together with long aggregations resembling *Oidium*, and puts forth a number of tufted growths extending downward in the liquid. Aerial hyphæ can be observed on the surface. At the same time a considerable sediment is formed, which, as already mentioned, mainly consists of yeast-like cells of various shapes and sizes. The growths obtained by inoculating beer wort from the film and sediment respectively, differ so greatly as to give rise to the impression that they are cultures of dissimilar organisms; and these variations remain constant for several generations. In respect of its physiological behaviour, *Monilia variabilis* belongs to the fermenting group of the genus, since it ferments glucose, fructose, galactose, trehalose, saccharose, lactose (doubtful), raffinose and dextrin, though leaving intact mannose, which, on the other hand,

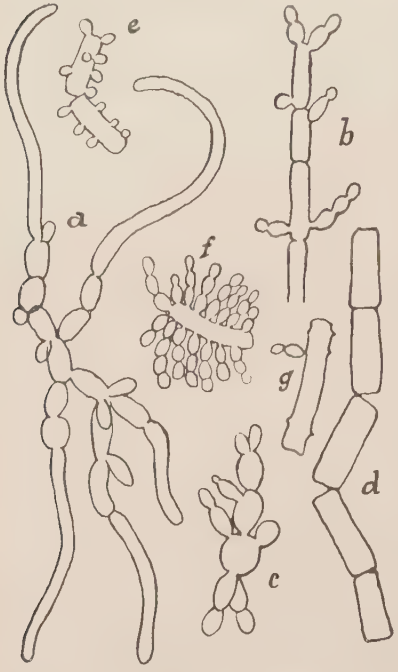


Fig. 203.—Monilia variabilis.

a, young bud mycelium, with terminal cells elongated to filaments; *b*, older filaments with yeast conidia; *c*, yeast-like bud mycelium; *d*, Oidium-like disintegration of aged hypha; *e*, Oidia with torulaceous conidia; *f*, same conidia germinating—"aerial cells"; *g*, Oidium, after shedding the conidia, showing basidial tubercles. Magn. 600. (*After Lindner.*)

is fermented by *Monilia candida*. Alpha-methylglucoside and β-methylglucoside are also fermented, which latter, according to LINDNER (XL.) is attacked by only one other micro-organism, namely, *Sachsia suaveolens* (*see* p. 335). HEINZE and COHN (I.) give *Monilia variabilis* as a true lactose ferment. In any case the production of alcohol is small, being only 1.4 per cent. by weight in beer wort after five months.

Despite their extensive fermentative capacity, the *Monilia* described above have no technical importance; whereas the two now to be mentioned find application in the preparation of foodstuffs in Eastern Asia.

Monilia javanica is the name given by F. A. WENT and PRINSEN-GEERLIGS (II.) to a fungus occurring, in association with others, in Ragi (*see* p. 67, vol. ii.). It forms dense filamentous masses, interspersed with, usually globular, cells (so-called yeast conidia). When grown on solid, artificial nutrient media, such as agar-agar or rice (*Oryza glutinosa*), the edges of the colonies exhibit septate filaments, on which account the discoverers of this species regarded it as the sterile condition of a higher fungus, a conclusion which more recent investigations have failed to confirm. The species thrives well in saccharine nutrient liquids, on which it first forms a film before commencing to produce alcohol—a circumstance pointing to very feeble initial fermentative activity. Glucose, fructose, saccharose, maltose and raffinose are fermented, but not lactose. Owing to the presence of volatile fermentation-products, the alcohol formed (maximum 5 per cent.) has a disagreeable flavour and smell, so that the arrack furnished by this fungus is of inferior quality. Dextrin and glycerine are also utilised as foodstuffs, the former being also fermented to some extent as well.

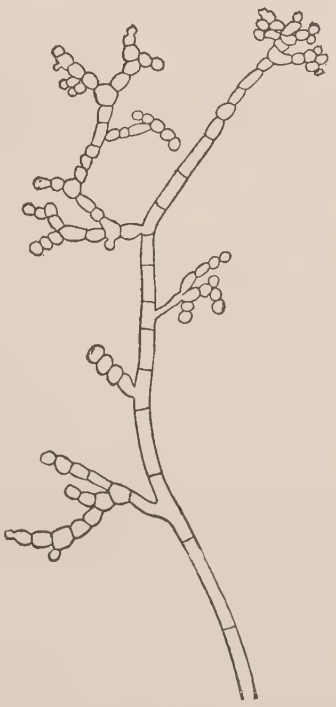


FIG. 204.—Monilia sitophila.

Aerial hyphæ (conidiophores) with budding yeast conidia. Magn. 220. (*After Went.*)

Monilia sitophila (Mont.), Saccardo, is said by WENT (IV.) to be used by the natives in West Java in the preparation of a sweetmeat known as "ontjom," composed of the seeds of the ground-nut or earth-nut (*Arachis hypogæa*). The ground-nuts, which are thoroughly permeated by the fungus, are made up in the form of small, orange-coloured cakes, the surface of which is covered with the conidia, whilst the interior is both chemically altered and loosened in structure by the mycelium. In the interior of solid media and nutrient liquids, the fungus develops into a plentifully branched mycelium of radial hyphæ, whilst the hyphæ projecting above the surface of the medium produce numerous conidia on short stalks. The oval to cylindrical conidia, measuring 5–14 μ, are produced by the formation of numerous septa in branches of the aerial hyphæ (regarded as conidiophores by Went), the individual cells thereafter becoming rounded and beginning to detach themselves. Simultaneously there occurs an additional reproduction, by the budding of the conidia, so that branched chains and groups of conidia are produced (Fig. 204). The conidia separate in a peculiar manner, recalling to

some extent the isthmus formation observed in the *Penicillia*. The conidia and that part of the mycelium which is in direct contact with the air, exhibit a pale orange-yellow coloration, due to a pigment similar to carotin. The colouring-matter is mostly distributed throughout the protoplasm as barely visible fine yellow drops, though sometimes collected into visible globules. In addition to the formation of conidia, Went also observed the production of small brown structures, comparable with young perithecia, but which could not be induced to develop completely. They commence with a spiral convolution of the hypha, which puts forth numerous branches and grows to a compact ball. The food requirements of *Monilia sitophila* have been thoroughly investigated by Went (V.). The fungus is very rich in enzymes, none of the more important ones being lacking, whilst even several of the rarer members of the group are present. Consequently, the organism was rightly looked on as omnivorous; and it is even able to thrive on filter-paper. It is almost insensitive to the general reaction of the nutrient medium, a high degree of acidity (10 c.c. of decinormal sulphuric acid per 100 c.c. of nutrient solution) alone being able to arrest development, whilst on the other hand large quantities of alkali have no important influence. The species grows vigorously even out of contact with air; and it is only when oxygen is completely excluded that it languishes. Small quantities of alcohol are formed in both anaerobic and aerobic cultures. One remarkable feature is the abundant formation of esters, which are also produced when protein constitutes the sole foodstuff. Went found this species growing on the spathes of sugar-canes in Java; and Saccardo observed it in wheaten flour and on dough at Lyons. Morphologically, it differs considerably from the other fermentation *Monilia*, and resembles the parasitic species (*Sclerotinia fructigena* and *Scl. cinerea*) in the structure of the mycelium and the restriction of conidia to the aerial hyphæ. According to Went, the enzymatic effects are the most important consideration in the technical utilisation of this fungus; the filaments bore through the cell wall of the ground-nut seeds, and loosen the cells so that they fall apart under gentle pressure, the proteids in the seeds are peptonised, the oil is decomposed, the small quantity of starch present is saccharified, and, finally, some slight importance must be attached to the esterification that is set up.

Monilia albicans (Robin), Zopf (X.), the pathogenic "thrush" fungus, which has also been described under the synonyms *Oidium albicans*, Robin, and *Saccharomyces albicans*, Reess, coincides exactly, morphologically, with *Monilia candida*. Possibly, the gemma-like formations observed by Grawitz might serve as a distinctive characteristic, as also the very moderate fermentative power, traces of alcohol being formed only after a very long time. This fungus is the cause of "thrush" on the mucous membrane of the mouth and throat in very young infants, puppies and kittens, as well as the corresponding disease in fowls; though it is probably associated with other organisms in these diseases. In Nature the fungus is of frequent occurrence on dead, rotting plants, and especially on dung, &c.

Both the fermenting and pathogenic species of *Monilia* are widely distributed, so that the technico-mycological literature contains numerous reports of forms resembling the species described above. Owing to their great morphological similarity, differentiation is often difficult; and owing to the omission of important morphological and physiological properties from the descriptions, it is seldom that the fungi described can be clearly identified with previously known species. On the other hand, many of the *Monilia* have probably been described by different authors as spherical yeasts, film yeasts, mould fungi, &c. The majority of the forms referred to simply as *Monilia* were found in wine, cheese, Chinese yeast, decaying fruit and concentrated fatty substances for feeding cattle.

Sachsia albicans, Bay, is the name given to a fungus accidentally discovered by J. C. Bay (IV.). On the surface of solid and liquid nutrient media it develops a snow-white mycelium, from which numerous cells, resembling those of *Mycoderma*, separate by constriction. When submerged, the mycelial buds bear a greater resemblance to *Dematium* or *Monilia*, and the detached bud cells are yeast-like, globular, ellipsoidal or pear-shaped (Fig. 205, *a*, *b*). Morphologically, this species coincides more nearly with *Monilia*, though the absence of alcoholic fermentation constitutes a difference that was pointed out by Bay himself.

Sachsia suaveolens, the mould fungus of wine bouquet described by P. Lindner (XL.), was discovered in the fermentation vessels of a distillery. It forms a brilliant white aerial mycelium on wort gelatin, whilst in wort it produces large flakes of threads that bud abundantly and readily fall apart into separate cells (Fig. 205, *c*, *d*), the whole of the wort being finally occupied almost completely with masses of the fungus. At high temperatures fermentation is set up at the same time, a very high final attenuation being eventually attained. An agreeable odour, resembling that of Moselle wine, is produced during the fermentation; but the flavour of the fermented liquid, which is rather acid, is too strongly aromatic to be pleasant. This species ferments glucose, mannose, galactose, lactose, maltose and dextrin, as well as raffinose and β-methyl glucoside. Mucinous masses are formed in some of the sugar solutions; and old cultures exhibit isolated greenish mycelial filaments, the cells of which contain large numbers of fatty drops. The faculty of developing a bouquet is utilised in the preparation of a non-alcoholic beverage, for which a patent was taken out by Mirsch and Eberhard (I.). This fungus closely resembles the *Monilia*, both in the development of the vegetative organs and in respect of its fermentative capacity.

Fig. 205.—Sachsia albicans and Sachsia suaveolens.

a, budding cells (magn. 775); *b*, normal mycelium (magn. 325) of Sachsia albicans; *c*, mycelial filaments of Sachsia suaveolens in the act of budding; and *d*, fully septated (magn. 300). (*a* and *b*, *after Bay*; *c* and *d*, *after Lindner*.)

Chalara mycoderma, a name first applied by Bonorden (I.), was afterwards bestowed by L. Cienkowski (III.) on a fungus discovered by him in the films on organic liquids (wine, milk, fruit juices, sauerkraut liquor, &c.). This species, which was afterwards discovered and depicted by E. C. Hansen (LIX.), is mentioned here in connection with the *Monilia* because the film it produces on the surface of nutrient liquids is similar to that of *Monilia candida*. The extensively branched mycelium of the former organism, however, consists of rafter-like aggregations of elongated buds, from which globular to ellipsoidal

conidia separate by constriction at the points of contact of the cells. These conidia, which measure about 4–6 μ, are often on short stalks or sterigmata; and they may also be developed on the individual cells into which the mycelium frequently disintegrates after the manner of Oidia. Being closely packed with protoplasm and therefore highly lustrous, these conidia are readily distinguished from the delicate, and apparently empty, elongated cells, so that *Chalara* presents a characteristic appearance under the microscope.

§ 315.—Oidium lactis and Allied Species.

The widely distributed *Oidium lactis*, the chief representative of the whole clan, is classified with the *Basidiomycetes* by the systematic mycologist, on account of the peculiar forms of vegetative reproductive cells known as *Oidia*, first observed in their fullest development in *Oidium lactis*. Their form, origin, and importance have already been mentioned on p. 18, vol. ii.; and it should also be stated that the rectangular contour of the conidia is so marked that it is very difficult to mistake an *Oidium* cell for any other kind. So long as the *Oidium* fruit cannot be satisfactorily assigned to the second typical, basidiomycetous fructification, these *Oidium* species may be grouped with the fungi imperfecti, since they are connected with many species of this group by transition forms. We have also retained the old and characteristic name, *Oidium*, because it is well known in all the literature of fermentation, including the present work, and because, in the present indefinite systematic position of the genus, it would be inadvisable to employ the proposed new name, "Oospora."

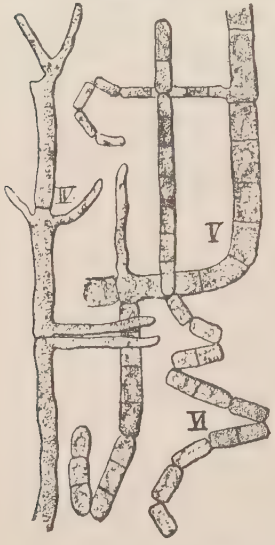


Fig. 206.—Oidium lactis.

IV, hypha with a number of lateral branches; *V*, a branched thread in course of septation; *VI*, disintegration into oidia, the chain bending into the zigzag form. Magn. 600. (*After Lindner.*)

The species of the genus *Oidium* are characterised by a typical mycelium, consisting of septated, irregularly branched hyphæ, which disintegrate—mostly at the ends, though sometimes in the middle as well—into short cylindrical cells of nearly rectangular contour, only the corners being rounded off a little. Budding is only exceptionally observed with this genus.

Oidium lactis, Fresenius, a fungus widely distributed in Nature and in the fermentation industries, is generally known as milk mould, being almost invariably found on sour milk, and also on the surface of unclean dairy utensils, cheese, &c. It is of such regular occurrence in butter that Laser (II.) proposed to utilise this circumstance as a biological test for that substance. Other common habitats of the fungus are the surface of packages containing pressed yeast, on pickled gherkins, commercial starch, green malt, and in breweries, where it occurs on the sludge-filter bags, wooden utensils, and storage casks (as a white efflorescence), also in waste waters, the dung of domestic animals, &c.

When viewed by the unassisted eye, the fungus has the appearance of a delicate, white down composed of fine threads, though sometimes it is mealy and dry—rarely yellow and mucinous—whereas in artificial cultures on various nutrient media it always forms a uniform snow-white, closely matted, furry covering. The appearance is exactly the same on nutrient liquids, so that an

Oidium culture can always be identified at the first glance. Old cultures exhibit isolated upstanding masses of hyphæ, resembling *Basidiomycetes*. Lindner (XL.) described similar forms, owing their origin to the fact of the film plectenchyma in giant cultures being perfectly gas-tight, and consequently forced upward, like bubbles, by the carbon dioxide liberated during fermentation, and being prevented from escaping at the sides owing to the colonies adhering firmly to the nutrient medium at the margin.

The mycelium in the fungoid mass, which is often extensive, consists of septated and very irregularly branched hyphæ, the members of which are comparatively long (Fig. 206). In young mycelia, especially during the germination of the conidia, the cells are tubular, septation only occurring later; and the conidia are difficult to distinguish from the short cylindrical cells. The conidia, which have the typical *Oidium* form, are developed most completely when one of the hyphæ raises itself above the level of the substratum and divides into short cells by septation, when growth at the apex is concluded. The various cells soon become detached, without becoming rounded to any extent, this separation being accompanied for the most part—as observed by Lindner—by spasmodic movements of the whole thread. In nearly all cases the separation is so far incomplete that two or more oidia hang together by a corner, and thus form zigzag chains. A filament in this state is particularly characteristic, though even when unbroken the conidial chains present a remarkable appearance owing to the uniformity of shape and dimensions of the members. After their detachment the conidia preserve their rectangular contour for some time, and become rounded only just before germinating. When these isolated oidia are mixed with yeast cells, *e.g.*, those of pressed yeast, they are readily distinguishable from the latter, both by their shape—the longitudinal walls are perfectly parallel—and contents, these showing up in strong relief and being interspersed with numerous vacuoles and granules. Moreover, the conidia are not merely produced on special hyphæ—this, indeed, being the exception—but the central portion of a hypha may frequently be observed to separate into oidia—especially on very moist nutrient media—and not infrequently the entire mycelium disintegrates into its component cells. This behaviour brings *Oidium lactis* and its congeners into close relation with *Monilia*. Young mycelia, placed in a little water and allowed full access to the air, exhibit in a very marked degree the phenomenon of cell fusion or "internal conidiation" (*see* p. 5, vol. ii.).

The physiology of *Oidium lactis* has been already commented on in several parts of the present work; and these remarks may be briefly summarised here. Although, in comparison with *Monilia*, *Oidium* may be considered as poor in enzymes, it nevertheless possesses a by no means unimportant fermentative activity, which is manifested by a considerable liberation of gas. The quantity of alcohol thus formed is, however, slight, merely traces being produced, according to Hansen (LVIII.), in beer wort and glucose yeast-water, Weidenbaum (I.) giving the amount as 0.6 per cent. in a fortnight, and Brefeld 1.2 per cent. in three months. Lang and Freudenreich (I.) found 0.55 per cent. in ten days or 1 per cent. in five weeks; the fermentation, according to these workers, proceeding less vigorously in solutions of saccharose or maltose than in those of glucose or lactose. No invertase could be isolated. Lactic acid (*see* p. 246, vol. i.) is oxidised by this fungus, the acidity of sour milk being thereby reduced. The presence of proteolytic enzymes is indicated by the liquefaction of gelatin, which is facilitated by an acid reaction, a peculiarity regarded by Weidenbaum as a means of differentiation from *Oidium* (*Monilia*) *albicans*. Lang and Freudenreich also state that a strong smell of soft (Limburg) cheese is developed in peptonised meat broth containing lactose and maltose, and that a considerable (probably complete) decomposition of casein is effected in sterile milk.

Henneberg's observation that *Oidium lactis* is injurious to yeast, the cells of which it kills, is probably attributable to this powerful action on proteids. On the other hand, *Oidium lactis* itself offers very strong resistance to external influences. For instance, Lang and Freudenreich report that growth is not appreciably retarded below 60° C. The statements of various authors on the influence of temperature, however, differ considerably, Hansen (LVIII.) giving 37.5° C. as the maximum and 0.5° C. as the minimum, whereas, according to Weidenbaum (I.), the optimum temperature of vegetation is 20° C. According to Hansen, the maximum temperature for film formation is 36.5°–37.5° C., and the minimum 3° C., which clearly expresses the character of *Oidium lactis* as a true film-producer. The species also seems to oppose considerable resistance to anti-

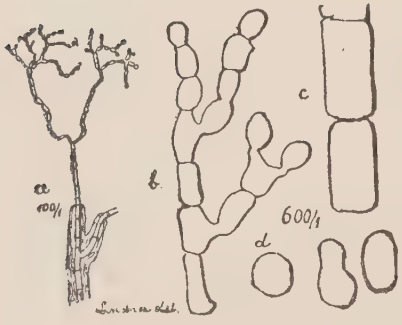


Fig. 207.—Oidium lupuli.
a, Aerial hypha, branched and separating into oidia; *b*, end of a branch of same; *c*, oidia from the central portion of a wide hypha; *d*, isolated oidia before germination. Magn. of *a*, 100; of *b*–*d*, 600. (*After Lindner.*)

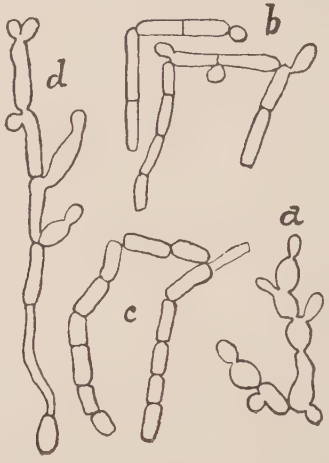


Fig. 208.—Oidium pullulans.
a, budding yeast-like cells; *b*, budding oidial cells; *c*, formation of oidia; *d*, monilial hypha. Magn. 600. (*After Lindner.*)

septics, even a 1 per cent. solution of corrosive sublimate being insufficient in some cases, whilst 1 : 1000 of formaldehyde failed, and 2.5 per cent. of carbolic acid required thirty seconds to prove fatal. Though growing well on all nutrient media, the fungus develops preferably on those with an acid reaction.

Oidium lactis is the representative of a group of species or varieties, a fact demonstrated by the divergent reports on the production of alcohol, the influence of temperature, &c. On the basis of morphological and physiological differences in the appearance of the colonies, in the size and shape of the oidia and in the power of peptonisation, M. Grimm (I.) set up a number of species, whose divergences are most clearly apparent in cultures on potatoes and casein.

Oidium lupuli, Matthews and Lott, is occasionally observed, as a reddish dust, on hops that have been stored damp, the dust consisting of the conidia of this fungus. In artificial cultures—according to Lindner (XLI.)—it forms a quick-growing superficial mycelium, the richly branched aerial hyphæ of which fall apart as oidia (Fig. 207). They are mostly oval, swelling up to a nearly globular shape before germinating. The colour is red at first, afterwards turning yellow.

P. Linder (XL.) also assigns to the genus *Oidium* a fungus discovered in samples drawn from the storage casks at the Berlin experimental brewery and

occupying a morphological position between *Oidium* and *Saccharomyces*. This species—*Oidium pullulans*, Lindner (*see* Fig. 208)—not only exhibits the oidium-like disintegration of the mycelial filaments, but also budding growths resembling yeast cells. In some small-drop cultures these yeast cells are formed exclusively, without any tendency to septation of the hyphæ, but in other cases there is an abundant formation of mycelial threads with budding cells. On wort it quickly forms a thin film with a strong ring; and on wort gelatin, yellow-brown colonies, resembling those of yeast and with a dull lustre. Lindner did not succeed in observing any fermentation. The close relationship of this fungus to *Monilia* is unmistakable, and it would probably be advisable to include it in that category.

SECTION XVII.

THE ENZYMES AND ENZYME ACTIONS OF YEAST.

CHAPTER LXIII.

ALCOHOLASE.

By Dr. RUDOLF RAPP,

Chief Pharmacist of the Munich Municipal Hospital.

§ 316.—Historical Introduction.

After the vegetable nature of yeast had been demonstrated by Cagniard-Latour (II.) and T. Schwann (I.), and the theory of its fermentative action had been thereby diverted into new channels, the succeeding decades witnessed the rise of a series of theories which endeavoured to assign the fermentative property of yeast to actions that were partly physical, partly chemical, and partly physiological. The most important of these theories have been already discussed in vol. i. (pp. 10–20).

The fact that, until recently, Pasteur's theory on this subject found the largest number of adherents was due to its being the most intelligently developed and to the circumstance that the systematic investigations of that authority first laid the foundations of the chemistry of fermentation. Moreover, Pasteur based his work on the labours of Schwann, whose disciple he professed himself—*see* Delbrück (VII.)—so that he was not really the founder of the new epoch.

In the present paragraph we will deal chiefly with the theories in which fermentation is ascribed to the action of enzymes, to which class belong the hypotheses of M. Traube, J. Liebig and Hoppe-Seyler. Traube (I.) wrote: "Yeast acts as a chemical ferment, which transmits the oxygen present in combination in one chemical substance to another substance," that is, it is capable of acting as a reducing agent on the one hand, and as an oxidising agent on the other. This view was quickly shared by M. Berthelot (V.) and was repeated by Traube (II.) in 1874, after Brefeld (XIII.)—on the basis of a somewhat doubtful method of experiment—had come to the not very expressive conclusion that yeast reproduction and alcoholic fermentation seem to be two distinct phenomena. As far back as 1839, J. Liebig (I.) classed fermentation and putrefaction as the results of the transmission of chemical motion, proceeding from a proteid ("ferment") substance in a state of incipient decomposition. At a later date (1870) this worker (II.) expressed the opinion that this chemical ferment was a nitrogenous and sulphurous body present in the yeast cell in a state of decomposition and transformation; and that the sugar of the fermenting liquid combined with the proteid substances in yeast to form a solid protein saccharate, alcohol being then separated in consequence of the rearrangement of one or more constituents of the ferment. A similar view was strongly supported by Hoppe-Seyler (VI.), who wrote: "Lower organisms are undoubtedly producers and carriers of ferments just the same as higher ones. In my opinion,

the possibility of the production of an alcoholic ferment by germinating yeast is established in principle by the separation of invertase from the yeast cell."

Although the existence of a fermentative enzyme was assumed by celebrated scientists, and the possibility of the production of such a substance by budding beer yeast was brought within the bounds of probability, for example, by the discovery of Fiechter (I.) that hydrocyanic acid destroys the vitality and development of yeast completely, though not necessarily its action as a ferment, an important difference still existed between the enzymes already known and that concerned in alcoholic fermentation. The former were able to perform their functions even when separated from the living cell, whereas the hypothetical yeast enzyme could not at first be isolated from the living cell. The question arose, given such an enzyme in beer yeast, why cannot it be isolated, and since experience teaches that enzymes act only when in a dissolved condition, why is the yeast cell alone able to set up the fermentative action? This question became particularly important when Berthelot and Liebig succeeded in isolating invertase from the yeast cell.

All the attempts made by workers at different times to separate an alcoholic ferment from the yeast cell proved futile. Thus, the experiments of Berthelot (V.), for recovering the enzyme from yeast by maceration, failed. Mitscherlich (IV.), Helmholtz (I.), Dumas (V.) and others filtered the yeast liquid, or tried to separate the yeast from the dissolved constituents by diffusion through a fine membrane, but in none of these cases was any fermentation set up so long as reinfection with yeast cells was carefully precluded. Even wort that is in full fermentation soon ceases to ferment when the yeast cells are removed by filtration and the access of new yeast cells is prevented. This experiment was recently repeated by Wroblewski (V.), who employed sandstone as the filtering medium. A different result was obtained by Colin (I.) and afterwards by Fleck (II.), but their experiments were valueless, not having been conducted under conditions guaranteeing sterility.

Other workers proposed a different method of separating the fermentative enzyme from the yeast cell, it being hoped that fermentation would be excited by the cell contents when liberated by the disruption of the cell membrane. Experiments on this line were undertaken by Lüdersdorff (I.) and C. Schmidt (at Dorpat), as also by M. Manassëin. The first-named triturated yeast on a ground glass plate by means of a glass pestle. C. Schmidt (I.) consumed six hours in grinding down 1 grm. of yeast, but failed to obtain any fermentation with the product. M. Manassëin (I.) attempted to kill the yeast cells used for the fermentation experiments by the aid of boiling temperature, without destroying the enzyme.

The fact revealed by all these communications is that the existence of an alcoholic enzyme in beer yeast could not be demonstrated by experiment, but that the presence and reproduction of living yeast cells are essential to the inception and continuance of alcoholic fermentation.

Though this state of things apparently controverted the hypothesis of the existence of a fermentative enzyme, further researches and observations contributed to prevent the enzyme theory from stagnating, the chemists, in particular, strongly maintaining the existence of an enzyme. The failure to isolate from yeast an active enzyme capable of decomposing sugar into alcohol and carbon dioxide was generally ascribed to the circumstance that the method of treatment pursued probably altered the composition of the enzyme and rendered it inoperative. Even if alcoholic fermentation had not yet become an accomplished fact, there was no justification for assuming that it would not some day be realised, when a method should be discovered of preparing this enzyme without impairing its activity.

These opinions on the existence of an alcoholic enzyme necessarily acquired a more stable foundation when P. Miquel (VII.), in 1890, succeeded in demonstrating that the fermentation of urea is effected by the intervention of an enzyme, urase (*see* vol. i., p. 257), separable from the bacteria present, and not by the vital activity of these bacteria themselves, and when E. Fischer and P. Lindner (II.) isolated from *Monilia candida* (*see* p. 331, vol. ii.), by triturating the cells with powdered glass, a substance capable of decomposing saccharose in a manner analogous to invertase. The probability of this view was further heightened when E. Fischer (V.) applied the stereochemical method to enzyme action and showed the previously assumed difference between the chemical activity of the living cell and the action of chemical agencies with regard to molecular asymmetry to be non-existent; additional confirmation being afforded in course of time by the growing number of communications to the effect that, under certain conditions, the formation of alcohol, either alone or associated with carbon dioxide, could be demonstrated in the case of higher plants and in fruits in the absence of yeast cells. Under this category may be ranked the publications of Schunck (I.) and Duclaux, the former of whom found that alcohol, succinic acid and carbon dioxide occur during the so-called fermentation of madder—though, as the author himself admitted, the collaboration of living micro-organisms was not precluded. The observation of Duclaux (XVIII.) respecting the production of alcohol in an alkaline sugar solution has been mentioned in vol. i, p. 20. We may also refer here to the observations of Will (XXXV.), who desiccated yeast at a low temperature and detected fermentation phenomena, but not growth, on examining the samples after nine years' storage.

Thus, during the prolonged discussion on the nature of fermentation previous to the year 1896, opinion had gradually veered round again in favour of the enzyme theory. The experimental proof, however, that the fermentative agent is separable from the living cell, and that consequently the decomposition of sugar into alcohol and carbon dioxide should be regarded as a purely chemical reaction, must nevertheless rank as a new and important fact. This proof was afforded in a simple and precise manner by E. Buchner (II.) towards the close of 1896, in that, by purely mechanical means, after breaking down and removing the cell membranes and the protoplasmal envelope, he obtained the cell contents by themselves, in the form of juice capable of decomposing sugar, and in a practically cell-free condition. Although this discovery has become of the greatest theoretical importance and roused considerable attention in the fermentation industry, it does not seem adapted for direct technical application, so long as the yeast cell has to be drawn upon as the source of the enzyme, owing to the roundabout character of the method. This view was also adopted by Wortmann (XVIII.) in connection with the fermentation of wine, in which the entire metabolism of the living yeast comes into play. E. Fischer (VI.) expressed great approbation of the discovery. The practical application of the method and the preparation of permanent yeast are described in § 320; and the method of preparing the yeast juice by crushing and pressing the cells has found suitable application through the labours of H. Hahn (II.), Mazé (II.), Takahashi (I.), Weinland (I.), Kohnstamm (I.), Czapek (V.), Stoklasa (III.) and his colleagues Cohnheim (I.), Krasnosselsky (II.) and Maximow (I.).

§ 317.—Preparation of Expressed Yeast Juice.

The method of preparing expressed yeast juice was originally elaborated at the Munich Hygienic Institute, valuable assistance being afforded by M. Hahn, who recommended the use of kieselguhr and the hydraulic press. The opera-

tion is divided into five stages: (1) Washing the beer yeast; (2) Draining the washed yeast; (3) Mixing with quartz sand and kieselguhr; (4) Grinding to a pasty mass; (5) Pressing—these two latter stages being repeated. On account of their importance several of these stages will now be described in greater detail.

The washing of the brewery yeast was found to be an essential feature. It is preferably performed in an apparatus designed by HAGENMÜLLER (I.). Before mixing, the yeast must be well drained to free it from water, for which purpose it is placed in a stout, unstiffened cotton cloth (*e.g.*, watertight tent canvas) and pressed for five minutes under a pressure of 50 atmospheres. The resulting yeast cakes, containing 70 per cent. of water, are next mixed with fine quartz sand (passed through a sieve with 200 meshes to the sq. cm.) and kieselguhr or diatomaceous earth, in the proportion of 1000 grms. of yeast to 1000 grms. of quartz sand and 200–300 grms. of kieselguhr, the whole being sifted through a coarse sieve (9 meshes per sq. cm.). The grinding is effected in small quantities—300–400 grms. at a time—either in a mill or in a mortar with a loaded pestle provided with a pestle guide, the operation being continued until a pasty mass is formed, that "balls" and becomes detached from the walls of the mortar. The pasty masses are united, placed in a damp press cloth, of the type mentioned above, and subjected to a pressure, rising gradually to 1280 lb. per sq. inch, in a hand-operated hydraulic press. In order to increase the yield, the press cakes are divided into small portions and triturated over again in the mortar. At first, water was added at this stage, but subsequently it was omitted as superfluous. The exuding yeast juice is allowed to trickle down on to a folded filter, and thence into a vessel cooled with ice.

The yield of expressed juice obtained by this method varies between 450 and 500 c.c., so that after deduction of the cell membrane about 60 per cent. of the total cell contents is recovered. WROBLEWSKI (V.) obtained a yield of 72 per cent.; MACFADYEN, MORRIS and ROWLAND (I.), working with a pressure of 200–350 atmospheres, obtained 5–87 per cent.; LANGE (II.)—with 15 per cent. of added water—44 per cent., and AHRENS (I.) 70 per cent. After this treatment the press cakes are not exhausted, a further quantity of fermentative material being recoverable by repeated trituration and pressing after a small addition of water. Indeed, according to BUCHNER and RAPP (VII.), these later fractions mostly exhibit a stronger fermentative power than those from the first operation. WILL (XX.) also states that the residual press cakes still contain considerable quantities of zymase. Presumably the fermentative enzyme is dissolved by a further addition of water.

When the expressed yeast juice is properly prepared, only a few unbroken yeast cells can be discovered in it under the microscope; but it may be stated at once that these cells are not capable of producing the fermentation phenomena exhibited by the expressed juice. The sediment is also free from micro-organisms after standing 3–12 days in the ice chest, nothing but a protein coagulum being discernible under the microscope.

A bacteriological investigation of expressed yeast juice by BUCHNER and RAPP (II.), yielded 50–100 bacterial cultures on meat-water gelatin, and 4 yeast colonies on beer-wort gelatin, per 1 c.c. of juice. The press cakes were microscopically examined by H. WILL (XX.), who found:

	Empty skins.	Bruised cells.	Intact cells.
In the first experiment .	55.0 per cent.	28.0 per cent.	17.0 per cent.
In the second experiment .	15.6 „	81.0 „	2.7 „

In the case of such an important new discovery, it is desirable to obtain confirmation by other workers; and this has been afforded by the labours of

H. Will (XXXV.), M. Delbrück (VII.), J. R. Green (II.), and Martin and Chapman (I.). After all these workers had obtained negative results, the three first-named were successful on a subsequent occasion, so that only Martin and Chapman really failed to obtain positive results. On the other hand, very satisfactory fermentations with expressed yeast juice were obtained by Wroblewski (I.), Ahrens (I.), A. Stavenhagen (I.), Macfadyen, Morris and Rowland (I.), and by Harden and Young (I.). Ahrens wrote: "When once we had acquired the technique of the method, we invariably obtained a highly efficient juice; and whereas, at first, we could not get any useful, positive results, we never failed afterwards."

In addition to the Buchner-Hahn method there are several others. In the first place, E. Buchner (X.) himself tried to recover the juice by the aid of liquid air, or by trituration with solid carbon dioxide, equal quantities of yeast and solid carbon dioxide being triturated for half an hour, and the liquid drawn off by aspiration. A yield of 20 per cent. was obtained. Albert (I.) also obtained a fermentative extract from permanent yeast by trituration with sand, kieselguhr and with an addition of water containing 10 per cent. of glycerine, followed by pressing. A new method of preparing yeast juice was worked out by Macfadyen, Morris and Rowland (I.), by subjecting the yeast to a number of rapid shocks in presence of particles of silver sand in a special apparatus. By this treatment none of the cells remain unbroken. The yeast was kept cool with the aid of brine at a temperature of -5° C. A yield of 35 per cent. of juice was obtained. Martin and Chapman (I.) recovered the juice by centrifugalising, instead of by pressure.

Other workers endeavoured to obtain extracts rich in protein or extractives, instead of those with fermentative power, and attained their end by means of energetic plasmolysis. Thus, C. J. Lintner (III.) recovered cell juice with salts, M. Hahn and L. Geret (I.) with chloroform, Dormeyer (I.) with ether and chloroform, and J. de Rey-Pailhade (V.) with 22 per cent. alcohol. In order to recover a yeast preparation similar to meat extract, E. de Meulemeester (I.) proposed to treat yeast with gum arabic; H. van Laer (IX.) used 2 per cent. of common salt, and H. Buchner and M. Gruber (I.) used ether. Yeast extracts of this kind are met with in commerce under various names: siris, wuck, ovos, &c. (*see* p. 122, vol. ii.).

§ 318.—General Properties of Expressed Yeast Juice.

The expressed yeast juice prepared by the method of Buchner and Hahn is a clear, though opalescent, liquid of a yellow to brownish yellow colour, and with a strong smell and taste of yeast. When freshly prepared, it has a faintly acid reaction, though Ahrens (I.) states that that prepared by him is slightly alkaline, but quickly turns acid. The fresh juice prepared by Wroblewski (III.) was also faintly alkaline.

According to this worker (IV.), the juice is optically inactive, which is rather surprising in view of the protein content. E. Buchner's (X.) investigations on this point have not yet led to any decisive results.

When the yeast juice is treated with strong alkali, mineral acids or acetic acid, a voluminous precipitate is formed. On being warmed, coagulation of protein occurs at 35°–40° C., becoming much stronger at higher temperatures, so that the whole mass seemed curdled. This is probably the first time that a preparation so rich in protein has been obtained from the cells of budding fungi. By means of partial coagulation, Wroblewski (III.) was able to differentiate several coagulable proteids. He points out that the temperature of coagulation (41° C.) of the one proteid coincides with that at which the fermentative action

of the juice ceases; and that furthermore the proteid coagulating at 41° C. appears to be digested before any of the others.

According to E. Buchner (X.), the chemical analysis of different samples of juice gave the following results: sp. gr. 1.027–1.057; content of dry matter 8.5–14.5 per cent., coaguable protein 5–6 per cent., total nitrogen 0.82–1.45 per cent., organic phosphorus about 0.228 per cent., organic sulphur about 0.065 per cent., ash 1.3–1.8 per cent. (*see also* p. 139, vol. ii.). The ash constituents are: potassium, sodium, calcium, magnesium, phosphoric acid, sulphuric acid, chlorine and silica (the latter from the kieselguhr). Ahrens (I.) found in the ash-free substance, 45.4 per cent. of carbon, 7.5 per cent. of hydrogen and 10.64 per cent. of nitrogen (*see also* p. 204, vol. ii.). Wroblewski (I. and III.) has also identified the following substances in expressed yeast juice: albumens, globulins, mucinous substances, proteoses, peptones, nucleoalbumens, compound carbohydrates and a special crystalline body that leaves behind, on combustion, a quantity of phosphoriferous ash. Other substances present are: tyrosin, leucin, glutamic acid, nitrogenous bases, xanthin bodies, a substance capable of changing sulphur to sulphuretted hydrogen and iodine to hydriodic acid; lecithin, glycerine, calcium phosphate, magnesium phosphate, peculiar volatile bodies and various others.

As was shown by Buchner and Rapp (V.), expressed yeast juice can be desiccated without injuring any of its properties. The best method is to concentrate it to the thickness of syrup in a Soxhlet vacuum apparatus, after which it can be brought to complete dryness by exposing it, in thin layers, to the air at a temperature of 22° C., or at 34°–35° C. In the case of Munich yeast juice, the fermentative power was unimpaired by this treatment, but, according to E. Buchner (X.), a loss of 18–74 per cent. in this respect is sustained by Berlin yeast juice.

The most interesting feature of expressed yeast juice is the enzymes it contains, which include: a fermentative enzyme, a hydrolytic enzyme (decomposing maltose, saccharose and glycogen), a proteolytic enzyme, an oxidising enzyme, a reducing enzyme, one that decomposes fats, another that splits up hydrogen peroxide, and a lab enzyme (*see* chapters lxv. and lxvi.). The most important is the one under whose influence sugar is decomposed into alcohol and carbon dioxide. According to the recent labours of Buchner and Meisenheimer (II.), lactic acid plays an important part in the decomposition of sugar, and must be regarded as an intermediate product. Both these workers and also Mazé (III.) attribute the fission of sugar in alcoholic fermentation—with the intermediate production of lactic acid—to the action of two different enzymes; and they give the name of yeast zymase to the one that decomposes the sugar into lactic acid, whilst they propose the name, lactacidase, for the enzyme that transforms the lactic acid into carbon dioxide and alcohol. The quantitative determination of the absolute amount of fermentative enzymes in expressed yeast juice has not yet been carried out. The only possible means, so far, of obtaining information in this direction is by comparing the fermentative value of two different juices under identical conditions. All samples of expressed yeast juice exhibit fermentative power, provided the yeast from which they were recovered possessed any fermentative enzyme at all or was able to store up such an enzyme even temporarily. The fermentative power and fermentative energy, however, differ considerably, and depend on the race of yeast, and on the conditions (*see* § 320) under which the yeast was treated for recovering the juice. The amount of carbon dioxide furnished by 20 c.c. of pressed yeast juice, in presence of 8 grms. of saccharose and 0.2 c.c. of toluene (as antiseptic), at the end of ninety-six hours at 22° C., is 0.7–1.87 grms. According to E. Buchner (X.), 10.5 c.c. of juice and 3.5 per cent. of a 60 per cent. solution of saccharose furnish up to 30 c.c. of gas, after ninety minutes at 28° C; that is to say, two and a half times the

volume of the original liquid. Other workers observed greater fluctuations in the fermentative power. Thus, WROBLEWSKI (III.) obtained 1.8–10 c.c. of carbon dioxide from 3.5 c.c. of juice, 14 c.c. of water and 3.5 c.c. of a 60 per cent. solution of saccharose. The results obtained by MACFADYEN, MORRIS, and ROWLAND (I.) with top-fermentation yeast are so irregular that—as these workers admit—no clear information is obtainable therefrom. More favourable results were obtained by HARDEN and YOUNG (I.) with similar fermentation yeast. BUCHNER and ANTONI (I.) found that fermentation with expressed yeast juice goes on with equal power in an atmosphere of oxygen or hydrogen. Expressed juice has been prepared in Munich from bottom-fermentation yeast by BUCHNER and RAPP (I.–VIII.); in Berlin by Buchner, Albert, Spitta, Meisenheimer and Antoni from the bottom yeast of three different breweries; by LANGE (II.) from pressed yeast; by R. GREEN (III.) from *Saccharomyces cerevisiæ* (Hansen); by TAKAHASHI (I.) from Saké yeast; by WROBLEWSKI (I., II., IV., V.) from commercial yeast and wine yeast, pure cultures of beer yeast and Okoci distillery yeast; by MACFADYEN, MORRIS and ROWLAND (I.) and by HARDEN and YOUNG (I.) from top-fermentation yeast.

Before proceeding to describe the other peculiarities of expressed yeast juice, it may be mentioned that, up to the present, all the observations on the extent to which the fermentative enzyme are influenced by various factors have been made with the juice alone and not with the pure enzyme itself. Consequently, the degree to which the results obtained has been affected, one way or the other, by the other constituents of the juice, must be left out of consideration.

Influences of this kind come into play during the storage of the expressed juice. BUCHNER and RAPP (I.) observed that the juice loses its efficiency in proportion to the length of time and increasing temperature of storage; and the same peculiarity was also noticed by R. Albert and by MACFADYEN, MORRIS and ROWLAND (I.). Storage in ice affords the best means of preserving the fermentative power of the juice. The loss of power is ascribed by E. BUCHNER (III.) to extensive alterations, such as are set up by the autodigestion of the juice (*see* chapter lxvi.); but another cause might be traced to the gradual development of an acid reaction, AHRENS (I.) having found that, in the course of a night, the acidity increases from 0.305 per cent. to 0.81 per cent. (expressed as lactic acid).

Both the digestive action and the production of acid may be largely prevented by desiccation (*see* p. 345). As a matter of fact, the stability of the dried juice is considerable, for, according to BUCHNER and RAPP (VI. and VIII.) no appreciable diminution of fermentative power can be detected at the end of a year; and these results were confirmed by R. ALBERT (I.). The retention of fermentative power by sterile permanent yeast will be mentioned on p. 354.

A similar autofermentation to that occurring in living yeast (*see* chapter lxv.), is also noticed in expressed yeast juice. It is attributed to the glycogen content of the yeast (*see* p. 124, vol. ii.). In conformity with WILL'S (XX.) discovery of the low percentage of glycogen in Munich pressed yeast, the autofermentation in Munich yeast juice is slight, amounting—according to BUCHNER and RAPP (VII.)—to not more than corresponds to 0.45 grm. of carbon dioxide per 100 c.c. of the juice. According to E. BUCHNER (VII.) it is greater in Berlin yeast juice, and corresponds to 0.40–1.10 grms. of carbon dioxide per 100 c.c. of juice. The maximum value—exceeding even the fermentation in presence of saccharose—was that obtained by MACFADYEN, MORRIS and ROWLAND (I.) with juice from top-fermentation yeast, namely, 65–900 c.c. of gas per 100 c.c. of the juice; unfortunately, however, the cause of this unusually extensive autofermentation was not further investigated. The result is out of harmony with those of E. BUCHNER (VII.), and is probably attributable to the presence of living

bacteria. In a subsequent investigation conducted by HARDEN and YOUNG (I), the autofermentation, though greater than in Buchner's experiments, did not exhibit such considerable differences as in those of Macfadyen, Morris and Rowland.

Divergent results have also been obtained by the various workers on filtering the juice through a bacterium filter. Whereas BUCHNER and RAPP (I.), and also MACFADYEN, MORRIS and ROWLAND (I.), observed merely a diminution in the fermentative power of the juice when forced through a Chamberland filter—especially in comparing the first and subsequent fractions—WROBLEWSKI (III.) found that this function of the juice was entirely destroyed by the treatment in question. Similar results were obtained by STAVENHAGEN (I.) after filtering the juice through a Kitasato filter. This effect is probably explained by the circumstance that bacteria filters do not permit the transfusion of proteids to more than a slight extent, if at all (*see* vol. i., p. 78).

Hence the filtration of expressed yeast juice through a very fine filter may result in a loss of proteids, and, therefore, of the fermentative enzyme. For this reason it is important to bear the initial fermentative power of the juice in mind during experiments of this kind; for, since a decrease of this property must be expected from filtration, the initial power must be very high if the juice is to exert any fermentative action at all afterwards. This is the sole explanation of the unfavourable result obtained by Stavenhagen. It should also be mentioned that BUCHNER and RAPP (I.), by using a Chamberland filter, obtained a yeast juice that was perfectly devoid of cells, but exhibited active fermentative power despite the entire absence of germs.

§ 319.—Changes set up in Expressed Yeast Juice by External Physical or Chemical Influences, or by Living Organisms.

The influence of temperature on alcoholic fermentation with expressed yeast juice was investigated by E. BUCHNER (X.), who found that the fermentative power was maintained longest at 5°–7° C., but that fermentation commenced soonest at 28°–30° C., the absolute maximum effect being obtained with a temperature of 12°–14° C. In repeating these experiments, MACFADYEN, MORRIS and ROWLAND (I.) observed that the fermentative efficiency is increased at higher temperatures; but they left the time factor out of consideration, confining their experiments to a period of only forty-eight hours.

Desiccated yeast juice, on the other hand, will stand higher temperatures without loss of fermentative power. Thus, according to BUCHNER and RAPP (VI.), yeast juice that has been dried very carefully may be heated to 85° C. for eight hours without suffering any considerable loss of power, and even at 97° C. the fermentative power is not entirely destroyed. BUCHNER (VIII.) states that the precipitate obtained with alcohol and ether (*see* p. 351, vol. ii.) continues to excite fermentation after being heated to 105°–110° C. for four hours; and the same authority (V.) says that the permanent yeast (to be described later on) does not entirely lose its power when heated to 110° C. for six hours in the air, or to 100° C. for eight hours followed by heating for ten hours at 110° C. in a current of hydrogen, though it is destroyed by exposure to 140°–145° C. for an hour.

On the basis of existing knowledge it might be presumed, *a priori*, that the dialysis of the fermentative enzyme through animal membranes would be difficult, if feasible at all; and as a matter of fact, BUCHNER and RAPP (II. and IV.) have established that the enzyme cannot be extracted from the living yeast by lixiviation, nor can any considerable proportion be obtained by dialysis through parchment paper. Similar results were obtained by R. ALBERT (I.) with

sterile permanent yeast, no fermentative enzyme being extractable with the aid of water or sugar solution unless the cell membranes had been previously destroyed. According to the newer researches of HARDEN and YOUNG (III.) and BUCHNER and ANTONI (II.) the juice is apparently inoperative at the end of forty-eight hours after having been dialysed through Martin's gelatin filter or in Gürber's apparatus at 0° C., though when united with the concentrated dialysate or with scalded press juice, it becomes active once again (its power being increased threefold, or even more).

According to BUCHNER and RAPP (VI.), centrifugalising the expressed juice effects no change in its fermentative power, the various layers of the juice being of equal power before and after the treatment, provided the temperature has been kept within normal limits during the operation.

Alcoholic fermentation is an exothermic process, the decomposition of sugar into alcohol and carbon dioxide being accompanied by a greater disengagement than is observed during the action of other enzymes. This subject has been dealt with by FITZ (XII.), BERTHELOT (VII.), BOUFFARD (II.), BROWN (VIII.) and RUBNER (III. and IV.). Whereas Brown and Berthelot merely calculate that the heat liberated in the formation of the alcohol and carbon dioxide exceeds that requisite for the splitting up of the dextrose, and give the heat value as 67 and 33 calories respectively, Fitz, on the other hand, established by direct experiment that the temperature of an 18 per cent. solution of sugar increased by 18° C. during fermentation, but that 6° C. of this increase was due to a positive increment of heat external to the actual process of fermentation. The same thing was clearly shown in a primitive experiment by E. BUCHNER (X.), and Bouffard determined the heat of fermentation in a litre of grape must as 23.5 calories. Brown, in repeating this experiment with malt wort, found the heat of fermentation to be 119.2 cal. per gramme of maltose, or 21.4 cal. when referred to the gramme-molecule for comparison with Bouffard's figures. Finally, Rubner worked out accurate methods of determination, and recommended, in the first place, a differential method for estimating the heat of combustion of a nutrient medium before and after the growth of germs, and, secondly, a direct method of determining the heat liberated during the continuance of the vital activity. He determined the heat of combustion of bottom yeast as 4475 gramme-calories per grm. of dry substance, and that of top yeast as 4554 gramme-calories. Further experiments with alcoholic fermentation gave the mean value 149.5 gramme-calories (12 tests) as the heat of fermentation per grm. of saccharose.

In all cases the action of the enzyme is dependent, in a high degree, on the chemical reaction of the test liquid. According to BUCHNER and RAPP (I. and II.), the fermentative action of expressed yeast juice is accelerated by an addition of small quantities of alkalis, such as potassium carbonate, disodium phosphate and alkali arsenites. WROBLEWSKI (III.) and also HARDEN and YOUNG (III.) have expressed themselves in a similar sense, the first-named attributing considerable importance to phosphates, both alone and in presence of acids or alkalis. According to the first-named worker (I.), an addition of 0.05 per cent. of hydrochloric acid or acetic acid is injurious to the fermentative action (*see also* p. 180, vol. ii.). Nitrous acid has a greater restrictive influence on cell-juice fermentation than any of its salts. E. BUCHNER (X.) found that acetic acid, tartaric acid, and especially lactic acid, had a less injurious effect, the initial diminution in the liberation of carbon dioxide disappearing when the experiment was prolonged for some time. In the case of added lactic acid (0.3 per cent.), the fermentative power was even higher than in the check experiments.

With regard to the influence of other salts on yeast juice fermentation (*see* p. 179, vol. ii.), E. BUCHNER and RAPP (III. and VIII.) ascertained, by means

of quantitative experiments, that 1 per cent. solutions of sodium chloride and ammonium chloride retarded the fermentative power to only a small extent, whereas corresponding solutions of the sulphates of soda, ammonia and magnesia had a more serious effect, and calcium chloride was highly injurious, whereas barium chloride of equal strength was innocuous, and in fact rather favourable. According to Buchner and Antoni (II.), manganese sulphate, aluminium sulphate, ferrous sulphate and cobalt sulphate are either inoperative or only adverse in their effect. Turning to organic substances, urea and glycocoll increase the fermentative power, but, on the other hand, antipeptone, hemi-albuminose and protalbuminose are directly injurious. The phosphates, and especially the secondary phosphates, are particularly beneficial in yeast-juice fermentation, an addition of 1–4 per cent. having good results. Wroblewski (V.) finds that the optimum dose of secondary phosphates is 1.25 per cent. The beneficial effect of these salts is increased by the presence of acids and alkalis; and Wroblewski (V.) credits these salts with protective properties, exercised by neutralising the added acids and alkalis, so that the phosphates guard the living cell from the attacks of acids and bases. Additional matter on this point is furnished by the newer researches of Harden and Young (II.), according to whom the alkali phosphate added to the yeast juice is no longer precipitable by magnesia mixture after the fermentation is ended—a behaviour indicating the formation of an organic compound of phosphoric acid in the juice. From experiments made by Buchner and Antoni (II.), it appears that the addition of lecithin has considerable influence on zymase fermentation; and these authorities state that the active principle may consist of organic compounds of phosphoric acid.

Buchner and Rapp (VIII.) report that an extensive liberation of nitrogen follows the addition of nitrites to expressed yeast juice. The process is a purely chemical one and originates in the action of the amino acids and other amino compounds of the juice on the nitrites. The same observation was reported by Wroblewski (IV.), who also found that an addition of 0.25 per cent. of sodium nitrite increased the fermentative activity of yeast juice.

Although the yeast juice of itself, and particularly in view of the large proportion of added sugar, is bound to restrict the development of micro-organisms, it has been considered advisable to add some antiseptic in carrying on fermentations with the juice. According to Buchner (X.), corrosive sublimate renders yeast juice very turbid and destroys the fermentative power; and it is stated by Buchner and Antoni (I.) that the same effect is produced by even a 0.55 per cent. solution of ammonium fluoride or sodium fluoride. Sodium azoimide (dose 0.36–0.71 per cent.) diminishes the fermentative power, whereas the converse result is reported of a 0.5 per cent. solution of quinine sulphate by Palladin (I.), Gromow and Grigoriew (I.) and also by Buchner and Antoni (I.).

It is well known that hydrocyanic acid temporarily arrests the activity of most enzymes completely, the effect passing off when the volatile acid has been expelled by passing a current of air through the liquid. This behaviour has also been observed by Buchner and Rapp (I.) with the enzymes of alcoholic fermentation. In the earlier experiments of these workers (1–4), in yeast juice fermentations, extensive use was made of arsenites (*see* p. 179, vol. ii.) as antiseptics, but these were afterwards abandoned. In this connection, Abeles has pointed out that substances entering into direct combination with the proteids of yeast juice —an observation previously made by Biernacki (I.)—lose their toxic properties toward micro-organisms, and that 2 per cent. of sodium arsenite is incapable of restricting either the growth or the fermentative power of the cells, a portion of the latter being still active after the fixation of the arsenite. In consequence of this observation the use of arsenites was abandoned, more particularly because of their irregular action on fermentation under certain conditions. For instance,

an addition of 2 per cent. of arsenite prevented fermentation by yeast juice when the yeast had been stored, or the juice had been dialysed or diluted, or finally when dried juice (prepared at 35° C.) was used. Buchner and Rapp (VII.) attribute this peculiar behaviour to the disappearance or diminution of the high molecular proteids, so that proteids afford a certain amount of protection against the injurious action of arsenite, the same effect being produced by sugar when added in considerable quantity with, or directly after, the arsenite. The addition of 5 per cent. of arsenite completely arrests the fermentative power of the juice.

The influence of formaldehyde was investigated by Wroblewski (IV.), who found that an addition of 0.05 per cent. reduced the fermentative action to a very low level, whereas Macfadyen, Morris and Rowland (I.) observed a favourable effect with an addition of 0.0005 per cent. Buchner and Antoni (I.) found that the fermentative power was reduced to one-fifth by 0.12 per cent., and to between one-third and three-fifths by 0.24 per cent. in the case of active juice. Wroblewski (IV.) also investigated the influence of hydroxylamine hydrochloride, and found that an additional 0.65 per cent. extinguished the fermentative power of the juice.

Other antiseptics that have found extensive use in fermentation experiments are chloroform, thymol and toluene (*see* p. 181, vol. ii.). Now chloroform, though applicable for this purpose, causes a slight premature separation of proteids. Thymol is better, but is surpassed by toluene, which latter has also been largely employed by E. Fischer and P. Lindner (II.). Both of them possess sufficient antiseptic power. R. Albert (I.) claimed that higher fermentation values are obtainable in presence of thymol than with toluene, but the accuracy of the previous statement was afterwards confirmed by Buchner (VII.). In the experiments of Macfadyen, Morris and Rowland (I.) on the influence of antiseptics on yeast-juice fermentation, the results were so contradictory in presence of sugar, that the authors admitted the desirability of further experimentation on this point, which task was afterwards undertaken by Harden and Young (I.).

Glycerine and saccharose in large quantities also restrict development, living organisms either dying off quickly or at least losing their reproductive power in strong solutions of glycerine. Buchner (X.), however, found that fermentation with yeast juice still continued vigorously, even when the total content of glycerine or saccharose attained 45 per cent.

The influence of various quantities of sugar on the progress of yeast-juice fermentation may also be dealt with in this place. Buchner and Rapp (VII.) found that the quantity of carbon dioxide liberated by yeast juice attained the maximum in presence of a large addition of sugar (30–40 per cent.). Conversely, a small quantity of sugar (5–15 per cent.) must be selected when the fermentation is desired to terminate early. Macfadyen, Morris and Rowland (I.) obtained diametrically opposite results, the erroneous character of which, however, was pointed out by Harden and Young (I.).

Finally, alcohol must be mentioned as an antiseptic, and also as a precipitant. Experiments in this connection with yeast juice were carried out by Herzog (I.), Wroblewski (IV.) and Buchner and Antoni (I.). The first and two last-named of these workers found that the fermentative action of yeast juice on sugar is diminished as the amount of added alcohol is increased. The fermentative power of the juice, however, was not finally extinguished by 15 per cent. of alcohol; and it would seem as though the limit of the production of alcohol in cell-less fermentation were higher than with living yeast cells (*see also* p. 175, vol. ii.). According to the researches of Wroblewski the addition of 10 per cent. of alcohol restricts fermentation, whilst 20 per cent. arrests it completely.

Alcohol has long been in use as a precipitant for isolating enzymes. Since, however, prolonged contact with alcohol must be avoided with the more stable enzymes (*e.g.*, invertase), caution is all the more necessary in the case of the more sensitive enzyme of alcoholic fermentation. According to ALBERT and BUCHNER (III.), the whole of the fermentative enzyme can be recovered, without loss, when the expressed yeast juice is treated with at least twelve times its own volume of absolute alcohol—or preferably with a mixture of 800 c.c. of absolute alcohol and 400 c.c. of ether per 100 c.c. of yeast juice—the liquid being aspirated off as rapidly as possible, and the precipitate washed with ether and dried over sulphuric acid in a vacuum desiccator. The precipitated fermentative enzyme is completely soluble in water containing 2.5–20 per cent. of glycerine. In order to retain the fermentative power of the precipitate intact, the simplest method is to suspend it in the solvent. The portion dissolved by the aid of water and glycerine can be reprecipitated with ether-alcohol, without greatly impairing the efficiency of the preparation. The proteids are, of course, the chief constituent of the precipitates, so that only an insignificant proportion of the total weight of the precipitate consists of fermentative enzyme.

When methyl-alcohol is used as precipitant (*see* p. 178, vol. ii.), the fermentative power of the precipitate is, strange to say, entirely destroyed. Ether by itself, as first pointed out by WILL (XXXV.), causes the production of a jelly, which is rich in the fermentative enzyme. Favourable results have also been obtained with acetone for precipitating the proteids of yeast juice. At first the present writer made the mistake of using an insufficient quantity of the acetone, at least a tenfold volume being requisite for throwing down the whole of the fermentative enzyme. According to the later researches of BUCHNER and ANTONI (II.) the precipitates obtained with smaller quantities of acetone are deficient in the phosphoric acid compounds essential to successful fermentation, whilst increasing the quantity of acetone causes an increased proportion of saline matter to be thrown down in the precipitate.

In addition to the foregoing reagents, BUCHNER (X.) employed ammonium sulphate and also cholesterin, according to the method of Brücke, as a precipitant of the fermentative enzyme of yeast juice. In the former case, however, no fermentation could be observed at all, and merely traces in the latter. AHRENS (I.) used zinc sulphate and alcohol, and WROBLEWSKI (IV.) ammonium sulphate, for the same purpose, but neither of them tested the fermentative power of the precipitates. Wroblewski employed partial precipitation, and obtained five precipitates with an equal number of filtrates. In this partial precipitation the proteids—which, as already mentioned (pp. 344, 345) are coagulated at different temperatures—are thrown down in an incomplete manner. Finally, R. GREEN (III.) also confirmed the fact that the fermentative enzyme is carried down with the precipitates produced in yeast juice.

Mention may be made in this place of the investigations carried out with diluted yeast juice. WROBLEWSKI (V.) ascertained that dilution is accompanied by an unexpected extensive weakening of the fermentative power, whilst MACFADYEN, MORRIS and ROWLAND (I.) found that the addition of an equal volume of water noticeably retarded the autofermentation of the juice, and that double the quantity arrested the liberation of gas almost completely. BUCHNER (VII.), however, in repeating these experiments with greater accuracy, obtained entirely different results, no perceptible decrease of fermentative power being obtained by diluting the juice with a fourfold volume of 9 per cent. sugar solution; whilst it was only on dilution with 2–3 volumes of water that a gradual diminution was noticeable, and even this did not exceed 20–25 per cent. of the total fermentative power. BUCHNER (X.), moreover, was able to observe the occurrence of fermentation, in certain circumstances, even when the juice had

been diluted twenty-five'old. According to the recent investigations of HARDEN and YOUNG (I.), the dilution of the juice obtained from top-fermentation yeast has only a very slight influence on its autofermentation.

The fermentative enzyme suffers injury through digestive enzymes. As already mentioned, the fermentative power of yeast juice diminishes rapidly during storage, a result attributed to the proteolytic enzymes of the juice (*see* chap. lxvi.). BUCHNER and RAPP (I.) have shown that when yeast juice is treated with trypsin, papayotin or pancreatin, it loses its fermentative power more rapidly than the check samples, either on account of the direct action of the digestive enzymes, or indirectly in consequence of the decomposition of the high molecular proteids that protect the fermentative enzyme.

One of the most essential conditions for the investigation of cell-less fermentation is, of course, to exclude all action on the part of living organisms. H. LANGE (II.) tried to ascertain how far the presence of yeast cells in the crude juice affects the development of fermentation phenomena, and found that even when the proportion was ten times greater than the normal, it was incapable of setting up fermentation with anything approaching the same degree of vigour in concentrated solutions of sugar. Similar results were obtained, in this connection, by H. WILL (XX.). BUCHNER and RAPP (I.) made intentional additions of yeast cells and stale juice contaminated with bacteria; but in no case did the fermentative effect surpass that of the fresh juice. It should also be mentioned that—according to the experiments of GERET (I.) and RAPP (II.)—sterile permanent yeast, and therefore also the fluid contents of yeast, possess certain bactericidal properties.

§ 320.—Buchner's Zymase or Alcoholase.

E. Buchner explained the fermentative effect of expressed yeast juice as being due to the action of an enzyme, which he proposed to call zymase. This was the name applied by BÉCHAMP (VIII.) in 1872, for the enzyme we now term invertase; whilst other workers regard the word zymase as synonymous with yeast enzymes in general. In order to prevent the misunderstandings likely to arise from this multiplicity of meanings, we will in future refer to the enzyme of alcoholic fermentation as "alcoholase."

At present nothing definite can be stated with regard to the chemical nature of alcoholase. This enzyme forms a merely insignificant proportion of expressed yeast juice. According to WROBLEWSKI (V.), it is colloidal. Certain other facts speak in favour of its proteid nature, whilst others again indicate a morphological connection. Since the enzyme has not yet been isolated in a pure state, we can only deduce its nature from experiments with yeast juice. The characteristic property is its ability to split up certain sugars—compare BUCHNER and MEISENHEIMER (II.). In the dried state it appears to be fairly stable. It is incapable of dialysing through the cell membrane. Under certain conditions it is not destroyed by heating. It is sensitive in variable degree toward chemical reagents, acids being injurious, whereas alkalis in small quantities are beneficial. It is sensitive toward alcohol, but less so toward alcohol-ether or acetone.

Besides expressed yeast juice we have another preparation of yeast suitable for the study of alcoholase and other yeast enzymes, namely, sterile permanent yeast. Ordinarily, the fermentation technologist applies the term permanent yeast to yeast prepared so as to enable it to be despatched to considerable distances; but the substance we are now considering must not be confounded with this. Owing to the difficulties in the way of investigators preparing yeast juice themselves, or obtaining it in large quantities for the purpose of further research into the nature of alcoholase, considerable value attaches to a preparation that

can be made by any one without any special appliances, and that is also very stable. Furthermore, permanent yeast presents many advantages over yeast juice as a material for the investigation of fermentation phenomena, inasmuch as the whole of the alcoholase can be recovered from the prepared yeast by a skilled operator. The presence of the uninjured cells gives rise to difficulty only in certain investigations, *e.g.*, the extraction of alcoholase; and in such cases the cells must first be opened by trituration with or without sand.

The basis of the methods of preparing permanent yeast consists in the elimination of water, which is effected either by careful drying or by means of chemical agents, such as alcohol-ether—R. ALBERT (I.)—or acetone—ALBERT, BUCHNER, and RAPP (I.). In the last-named method the operation must not be regarded as one of plasmolysis, effected by the reagent, but as an extraction of moisture, the reagent penetrating the cell membrane and the strata of protoplasm, whereby all chemical reactions are arrested.

The acetonised permanent yeast, prepared from bottom-fermentation beer yeast, and known in commerce as zymin, is a white and practically sterile powder, as dry as dust, and containing 5.5 to 6.5 per cent. of water. The fermentative capacity calculated for 2 grms. of the preparation, disseminated in 10 c.c. of water, with 4 grms. of saccharose, and 0.2 c.c. of toluene as antiseptic, fermented for seventy-two hours at about 22° C., corresponds to 0.96 1.09 grm. of carbon dioxide. According to ALBERT, BUCHNER and RAPP (I.), the fermentative power amounts to 0.40–0.49 grm. of carbon dioxide in the first twenty-four hours, 0.36–4.45 grm. during the second similar period, 0.07–0.17 grm. in the third period and 0–0.02 grm. in the fourth. GROMOW and GRIGORIEW (I.) found that the addition of fresh zymin to a sample that has already become enfeebled causes renewed liberation of carbon dioxide to a greater extent than would be the case if the two quantities of zymin had been employed together at the outset. Hence the work of the newly added zymin is facilitated by the fermentation products of the amount employed at first. The preparation of acetonised permanent yeast is protected by patents; and the product itself is obtainable from Anton Schroder, of 45 Landwehrstrasse, Munich. It has found application for medicinal purposes, and has been used experimentally for baking by KOMERS and E. von HAUNALTER (I.) and for the detection of sugar in urine by MÜNZER (I.).

Sterile permanent yeast is suitable for further investigations on alcoholase. On this account mention may be made in this place of a series of questions, such as the amount of alcoholase present in yeast, and the formation, stability, accumulation and isolation of this enzyme.

Of these, the content and formation of alcoholase in yeast interest us more particularly. For investigations of this kind, permanent yeast has proved especially adapted, since it enables the total content of alcoholase to be ascertained, both previous to fermentation and at any other stage. Observation has long since demonstrated that the amount of alcoholase present in yeast is a factor varying with the physiological condition of the latter. On this point, WILL (XXXV.) expresses himself as follows: "It is possible that, like the peptonising enzyme, zymase is present only under certain conditions; and it is conceivable that yeast which has settled down after primary fermentation, filled with reserve substances and in a certain state of quiescence, contains very little zymase, if any." According to LANGE (II.) the alcoholase content is dependent to some extent on the nitrogen content (*see* p. 191, vol. ii.), which latter is stated by HAYDUCK (V.) to be a measure of the fermentative power of the yeast. GREEN (III.) also found that the formation of alcoholase is an intermittent function; and this view is confirmed by the results of the different workers who obtained expressed yeast juice with little or no fermentative power. The behaviour of yeast that will no longer excite normal fermentation in beer wort

after being used a certain number of times is also attributable to this cause. All these facts clearly indicate that the alcoholase content, and therefore the fermentative power, of yeast necessarily vary with the age of the yeast and divers other circumstances as well as with the character of the organism. This is a point that should be borne in mind in further investigations, the more readily so because the preparation of acetonised permanent yeast has afforded us a reliable means of arresting and determining at any stage all the reactions proceeding in the cell.

The alcoholase content of yeast during storage at low temperatures, under ice water, and in the regenerative process, has been traced in this way. The present is a most appropriate moment for devoting closer attention to the regenerative process of Hayduck (VI.). Previous to the time of that worker, brewers had frequently noticed that yeast after repeated use gradually ceased to give a satisfactory "break" (*see* p. 136, vol. ii.) and furnished a less compact sedimental yeast (*see* p. 168, vol. ii.). Hayduck (V.), uninfluenced by E. C. Hansen's recent discovery of the existence of a multiplicity of yeast species, many of them capable of causing haze in beer, attributed one cause of yeast degeneration (*see* p. 197, vol. ii.) to a surfeit of nitrogenous food (p. 157, vol. ii.), having traced, by analysis, the growing nitrogen content in the dry residue during the repeated employment of the yeast in brewing practice. In 1884 he attempted to lessen this injurious surplus by allowing the yeast to develop in a vigorously aerated wort at a higher temperature than that of the fermentation room, though not so high as to check reproduction. Then, stimulated by the favourable results obtained independently by a brewer, whose name has not transpired, Hayduck (VI.) replaced wort by a boiled solution of sugar (hopped in order to suppress bacteria) which, when vigorously aerated, gave a yeast crop comparatively poorer in nitrogen. In the present more complete state of knowledge respecting the nature of fermentation disturbances, there is no need to labour the point that this treatment might occasionally increase the quantity of any pre-existing disease yeasts in the sample, and certainly could not preclude that possibility. The method did not find any practical application. Nevertheless, it is worthy of mention, since, though as stated above it might occasionally lead to highly undesirable results, and must therefore be regarded as unreliable and defective in principle, it revealed a noteworthy fact, namely, that the yeast treated in this way was rendered more efficient as a fermentative agent than it had been previously. This was confirmed by R. Albert (II.), by preparing and comparing the effect of expressed juice from samples of yeast before and after regenerative treatment. Additional confirmation was supplied by E. Buchner and A. Spitta (I.), on repeating the experiment, acetonised permanent yeast being found more advantageous than yeast juice. From these results it seems probable that the stock of alcoholase in the yeast cells is low during the period when the "head" on the fermenting wort is most abundant, the alcoholase being apparently destroyed as it is formed, and not accumulated. After the yeast has been stored at a temperature of 0° C. the fermentative power is increased by 21 per cent. in three and a half hours, and 17 per cent. in twenty hours. It has also been found that yeast stored under ice water for twenty-four hours does not show any increase or diminution in the quantity of alcoholase present.

According to E. Buchner (X.), regenerated yeast is not that which contains a large store of alcoholase, but such as is capable of producing this enzyme quickly. If the yeast possessed a high initial fermentative power, one can hardly expect any increase from regeneration and storage. The product furnished by regeneration with a 20 per cent. solution of sugar was not particularly good in comparison with that resulting from the use of an 8 per cent. solution. The addition of 1 per cent. of asparagin to Hayduck's solution

containing no nitrogenous matter led to a slight diminution in the alcoholase content of the regenerated yeast, without any subsequent recovery during storage. According to the more recent researches of LANGE (III.), however, the fermentative power of yeast juice can be increased as much as ninefold if the yeast, before trituration, be immersed in a solution of saccharose containing asparagin as the chief adjunct. From this it appears that asparagin favours the production of alcoholase in the living cell. Further experiments on this point are highly desirable.

Whereas, in the experiments with yeast juice, the stability of the alcoholase was found to be very low, the fermentative power remained practically unimpaired at the end of twelve months in the case of specially well-dried juice (*see* p. 346). According to ALBERT, BUCHNER and RAPP (I.) a similar result was obtained with acetonised permanent yeast, the fermentative power of which decreased only by 10–19 per cent. at the end of six months' storage in tightly stoppered bottles at room temperature. Possibly the tendency toward loss of germinating power could be still further prevented by diminishing the content of water.

BUCHNER (III.) attributes the diminution of fermenting power to the action of endotryptase, which is formed in the yeast cells under certain conditions, but may also disappear again. A high temperature seems to assist the development and action of this enzyme. Up to the present it has not been found possible to separate alcoholase and endotryptase, both enzymes being apparently acted on equally by favourable or unfavourable influences, and on this account several points still remain unsolved. A. HARDEN (I.) found that serum strengthens the fermentative power of yeast juice, and observed at the same time a retarding influence on endotryptase (*see also* the remarks on quinine sulphate, p. 349).

The isolation of alcoholase is a highly important question. When it is remembered that none of the other enzymes has been completely isolated up to the present, a satisfactory solution of this problem can hardly be expected in view of the short time that has elapsed since the discovery of alcoholase. Nevertheless, a certain degree of progress has been made, especially when it is remembered that a highly labile substance is in question. In this connection AHRENS (I.) succeeded in increasing the fermentative power of yeast juice by freezing out the water. Other experiments in the same direction relate to precipitation with alcohol, and more particularly with alcohol-ether or acetone. AHRENS (I.) cooled the juice down to $-2°$ C., and obtained by this means a loose mass of ice, which consisted chiefly of pure water and was separable from the liquid constituents. By repeating this treatment a product of increased fermentative power was obtained, and MEISENHEIMER (I.), working on the same lines, found that the increase amounted to about 48 per cent. in the lowermost (5th) stratum. The precipitation of yeast juice by alcohol-ether or acetone has been already mentioned on p. 471; but this treatment does not effect any concentration of the alcoholase, the whole of the proteids being thrown down at the same time. The question whether fractional precipitation would furnish the desired result was examined by ALBERT and BUCHNER (III.), who found that while the first precipitation with a little alcohol throws down the bulk of the proteids, the enzyme is also deposited, and the second precipitate, with a larger amount of alcohol, is devoid of fermentative power. Experiments of this kind suffer from the circumstance that the precipitates are only very gradually soluble in water; and whilst it is true that solution is facilitated by an addition of glycerine, the resulting advantage is slight, since all the admixtures present are thrown down during the reprecipitation, and consequently no concentration of the enzyme is secured. The same thing happens when acetone is used as precipitant. A more favourable prospect was afforded by the experiments of

R. Albert (I.), performed with extracts from sterile permanent yeast. Since this material still contains the unbruised cells, and therefore the enzyme cannot be extracted under such conditions, the cell walls and envelope of coagulated protoplasm must be broken down before proceeding to the operation of extracting the enzyme with glycerine and water. Albert (I.) therefore allows 50 grms. of the permanent yeast to dry along with 100 grms. of quartz sand, and then triturates the mass with 100 c.c. of water, the liquid portion being afterwards separated from the solids by means of hydraulic pressure or an aspirator. Precipitation with alcohol ether furnishes 2–3 grms. of a yellowish white powder, which differs from the yeast juice precipitates in being readily soluble, and of being precipitable again and again without appreciable loss of fermentative power With regard to these experiments, all that need be mentioned is that the use of quartz sand in triturating permanent yeast is not free from objection, since dry grinding for ten minutes results in a noticeable diminution of fermentative power. Nevertheless it is probable that further progress toward the isolation of alcoholase may be accomplished in this manner, especially if loss of fermentative power be still further prevented by discontinuing the use of quartz sand, and if alcohol-ether be replaced by mixtures of acetone and ether or other innocuous precipitants, provided no very high degree of purity is expected in the resulting preparation.

The proof that the fermentative enzyme cannot be extracted from sterile permanent yeast, unless the cells have previously been ruptured by mechanical means, also demonstrates clearly that fermentation goes on inside the yeast cells, and not externally. This also follows from the circumstance that alcoholase cannot be dialysed, and that glycogen is not fermented by beer yeast until the cell membranes have been broken. Hence, alcoholase is an endoenzyme.

§ 321.—The Position of Alcoholase with Relation to the other Enzymes.

Before considering the relative position of alcoholase to the other enzymes, we will devote some attention to the discussions that have attended the discovery of this enzyme. So long as the separation of the fermentative enzyme from the living yeast cells had not become an accomplished fact, differences of opinion between scientists were readily conceivable; but even after the result in question had been achieved, an experimental solution afforded of the highly important problem, and all the errors of reinvestigation corrected, the doubting spirits began to advance other objections. At first it was sought to ascribe the fermentation to micro-organisms still present in the juice; but this objection fell to the ground when active antiseptics were used in all cell-juice fermentations, and after Lange had shown (II.) that the fermentative effect of the juice could not be produced by ten times the number of yeast cells found in the crude juice. Others put forward the opinion that the liberation of carbon dioxide was due to the fission of the plasma, or other causes. Another view that soon obtained prominence and found many supporters was that the fermentation resulted from the insignificant quantity of living plasma still present in the juice—a conception by no means novel in connection with enzymes. Kupfer and Voit (I.), soon after the discovery of cell-less fermentation, expressed the opinion that the same was *probably* due to fragments of protoplasm; and Abeles (I.) spoke positively in favour of this hypothesis after advancing several proofs in favour of the theory. The same view was shared by Macfadyen, Morris, and Rowland (I.), Beijerinck (XXVII.), Wehmer (XXXIII.), Behrens (XVII.), C. J. Lintner (VII.), Soxhlet (II.), Iwanowski and Obrastzow (I.), and H. Fischer (I. and II.). On the other hand, Duclaux (XXI.), R. Green (IV.), Rey-

Pailhade (V.), Pfeffer (VII.), A. Richter (II.), and A. J. J. Vandevelde (II.) supported the purely enzymatic theory of fermentation.

At present we will only refer to the views of Abeles and of Macfadyen, Morris, and Rowland. Abeles (I.) says: "The fermentative power is dependent on the total dissolved, or more properly speaking suspended, organic mass contained in the yeast juice." Albert and Buchner (I.) showed, on the contrary, that a constituent precipitated from the yeast juice still possesses fermentative power when redissolved. If it be urged, on the other hand, in support of Abeles's plasmal theory that yeast reproduction occurs despite the plasmal poison, Abeles correctly points out that the toxic action on organised ferments depends less on the concentration of the poison than on the quantitative ratio between protoplasm and poison. The careful experiments of Buchner and Rapp (VI.) demonstrated that antiseptics which, like toluene and chloroform, do not enter into direct chemical combination with the proteids of yeast juice, will suppress the fermentative action of even large quantities of living yeast cells, and that the carbon dioxide liberated in these circumstances is formed entirely by the amount of stored-up alcoholase left out of consideration by Abeles. The last-named also stated that young cells in particular effect the fermentation of sugar solution (as observed by Wiesner thirty years before) after desiccation and even after exposure to 100° C. for several hours, the cells also retaining their reproductive capacity. Buchner in his heating experiments invariably found that the yeast cells were killed during the process. Macfadyen, Morris, and Rowland (I.), and also Wroblewski (V.), observed that twofold dilution of the yeast juice practically arrested the fermentative power; and they consider that this behaviour is so greatly opposed to that of enzymes under the same conditions as to constitute a serious objection to the enzyme theory accepted by Buchner. However—as mentioned on pp. 346, 347—this result was not obtained in the experiments of Buchner (VII.) or in the more recent ones of Harden and Young (I.). Finally, several physiological reasons may be advanced against the plasmal theory. For instance, the acceptance of this hypothesis affords no explanation of the circumstance that juice incapable of producing fermentation should occasionally be furnished by exceedingly vigorous yeast, although the hypothetical particles of plasma are present in the juice, or of the fact that the fermentative enzyme can be concentrated as was shown by R. Albert (II.), Buchner and Spitta (I.) and Lange (III.). In such case we can hardly assume that an alteration of the whole protoplasm has occurred.

In view of all these facts, and also of the behaviour of yeast juice in presence of toluene and 40 per cent. sugar solution, after centrifugalising, treatment in the Chamberland filter, desiccation, storage, heating, precipitation, extraction of the precipitate, extraction of killed permanent yeast with glycerine and water, and the reprecipitation of such solution, Buchner rightly concludes that no living agent is present in yeast juice. Those holding an opposite opinion will be obliged to furnish a new definition of what is meant by "life"—a course leading merely to useless polemics. It is an indubitable fact that yeast juice does not represent the cell in its entirety, but the cell contents freed from membrane and other insoluble constituents, that is to say, a product forming only part of the erstwhile living cells. To assume that, under certain conditions, the parts that have been separated from the living cells and are totally incapable of growth will be able to continue living, is a novel and highly improbable idea. On the other hand, the hypothesis that these separated, soluble components of the cell have retained their activity and are capable of exerting it under certain conditions, accords with all existing experience and observation, and appears to be correct. Moreover, it has been demonstrated that the fermentative agent forms only an insignificant proportion of the soluble cell-

substance, that it is soluble in water; and that it can be precipitated from triturated permanent yeast, redissolved and reprecipitated—properties that had never been expected of living protoplasm.

We may now proceed with the comparison between alcoholase and other enzymes. In the first place it must be remembered that the properties of all known enzymes vary more or less considerably, so that it is by no means surprising to find that the more labile alcoholase exhibits points of difference from other enzymes, which differences do not justify denial of its enzymatic nature. Alcoholase exhibits the same properties as other enzymes, including solubility in water, with or without glycerine; precipitability by alcohol, ether, or acetone; the faculty of being carried down with other precipitated substances, such as calcium phosphate, precipitated protein, &c., and finally, susceptibility toward chemical reagents and protoplasmal poisons. Differences are exhibited to some extent in respect of its incapability of dialysing, except with difficulty or under certain conditions; in its greater susceptibility to high temperatures—though this is less noticeable in the dry state than when in solution—in which particular it is on a level with urase and the inverting enzyme of *Monilia candida;* and also with regard to the merely occasional appearance of the enzyme in the living cell—in which respect, nevertheless, analogies can be found in the vegetable kingdom. The only important difference, however, consists in the greater amount of heat disengaged by this enzyme, and the slowness of its action. Neumeister (I.) is firmly convinced on this account, that the question is one of collaboration between various substances outside the living cell, which substances have retained the powers they originally possessed in the protoplasm.

The next question that arises is the allocation of alcoholase to its proper subgroup among the enzymes, and also whether it may be present as zymogen in the yeast cell. Buchner (X.) proposes to class alcoholase as the representative of a new sub-group—fermentative enzymes—of the large class of enzymes, whilst Duclaux (XXII.) ranges it with the enzymes of nutrition, and Wroblewski (V.) places it in the third group of catalysers, which are closely allied to the morphotic constituents of protoplasm. E. Buchner does not assume the presence of zymogen in the yeast cell, but Wroblewski (V.), on the other hand, is partly in favour of the existence of such a body.

The method employed in the preparation of sterile permanent yeast may also be applied, with advantage, to other ferments. In this way E. Buchner and J. Meisenheimer (III. and I.) have investigated the enzymes of fermentation by fission fungi, namely, lactic and acetic fermentations, as well as by *Monilia candida* and a lactose yeast; and the same course was adopted by F. Rothenbach and L. Eberlein (I.) with *Bacterium Pasteurianum*. In all these cases, fermentation, or the production of the corresponding acid, was obtained by using sterile preparations of this kind. The same method can also be applied with higher plants, especially when labile enzymes are in question. An enzyme similar to alcoholase was found by Stoklasa, Jelinek, and Vitek (I.), and Stoklasa and Simacek (I.) in sugar beet, peas, potatoes, flowers, meat, and lung tissue; by Marpmann (VIII.) in honey; by Simacek (I.) in pancreas; and by Arnheim and Rosenbaum (I.) in pancreas, muscle, and liver. Living yeast is often preferably employed in dealing with questions of a general nature relating to enzymes; and it is probable that the new preparation, zymin (*see* p. 353), may be suitable for these investigations, on account of its excellent keeping properties, ease in weighing, and definite fermentative power. Indeed, experiments of this kind have already been conducted with zymin by Palladin (I.), Telesnin (I.), Gromow and Grigoriew (I.), Herzog (III.), and Euler (I.)

CHAPTER LXIV.

THE CHEMISTRY OF ALCOHOLIC FERMENTATION.

By Dr. ARMINIUS BAU,

Chemist to the Kaiserbrauerei, Bremen.

§ 322. The Chemistry and Chief Products of Alcoholic Fermentation.

As already remarked in the introduction to vol. i., it must have been noticed, at a very early date, in connection with the production of wine, mead, and other alcoholic beverages from must, diluted honey, and similar raw materials, that the original sweet flavour disappeared, a frothy head being formed and a gas disengaged; and that the effect of the fermented liquor on the human organism was quite different from that of the fresh grape juice or sweet solutions of other substances employed. In spite of this, however, the changes occurring during fermentation long remained in obscurity; and the first researches in this direction were devoted to the origin of the alcohol, rather than to the remarkable formation of the frothy head or the liberation of the pungent-smelling gas. When the Alexandrian school had improved the originally primitive apparatus of distillation, experiments were made in distilling wine, and a product was obtained exhibiting the intoxicating properties of that beverage in an intensified degree. The discovery and preparation of alcohol resulted from the invention and elaboration of methods of distillation. A description of these latter can be found in the commentary on the works of Democritus—who was presumably one of the earliest alchemists of whom we have any knowledge—published by Synesios, who studied in Alexandria at the beginning of the fifth century. It is known that wine was distilled as long ago as the eighth century of our era, spirit of wine having been referred to in the writings of the alchemist Geber. It is also probable that early attempts were made to concentrate and rectify the aqueous distillate from wine by redistillation; and the Spaniard, Raymundus Lullus (1235–1315), found that this result could be effected by treating the distillate with caustic potash, followed by redistillation. About the year 1413, Basilius Valentinus wrote a clearer description of the method for obtaining a more highly concentrated product; but it was not until 1796 that the Russian chemist, Tobias Lowitz, succeeded in preparing anhydrous alcohol by combining the use of hygroscopic agents with fractional distillation.

On account of its origin, alcohol—which name is derived from the Arabic—was known as spiritus vini (spirit of wine) or spiritus vitis (grape spirit). The scientific name of this fermentation product is ethyl alcohol, or, according to the newer terminology, methyl carbinol. The chemical formula is CH_3–CH_2.OH, the methyl group CH_3 being saturated with the carbinol CH_2 (OH), or, as regarded from another point of view, the ethyl group C_2H_5 with the hydroxyl (OH). Ethyl alcohol is generally termed alcohol, for short, so that when this latter name is encountered in the literature, ethyl alcohol is always implied. In all other cases, the special nature of the alcohol is indicated by a prefix, *e.g.*, methyl, propyl, butyl, &c.

The manner in which alcohol is formed remained unknown until J. J. Becher, in 1669, expressed the opinion that saccharine liquids alone are capable of fermentation. The nature of the gas liberated during the process also remained unknown for a long time. It is true that B. van Helmont (1577–1644), who was a chemist as well as a physician, again pointed to the fact that a liberation of "gas" occurs during alcoholic fermentation. Nevertheless we are unable to gather from his work, *Ortus medicinæ vel opera et opuscula omnia* (first printed in 1648) whether he was really aware of the nature of the gas in question; and it was left for MacBride, in 1764, to correlate the gas of fermentation with the gas carbonum, or gas sylvestre as van Helmont called it, and to establish its identity with carbon dioxide (CO_2).

With regard to the quantitative yield of the main products of fermentation, Cavendish found the amount of carbon dioxide formed equal to 27 per cent. of the sugar decomposed. Lavoisier (II.) then attempted to determine the quantitative yield of both products, and obtained from 1 cwt. of sugar 35 lb. 5 oz. 4 dr. 19 grs. of carbon dioxide and 57 lb. 11 oz. 14 dr. 19 grs. of anhydrous alcohol, 4 lb. 1 oz. 4 dr. 3 grs. of sugar remaining undecomposed. Expressed in percentages these results gave 36.8 per cent. of carbon dioxide and 60.1 per cent. of alcohol. The fact that these values differ from those obtained at a later date is not surprising, because, on the one hand, analytical methods were not so well developed then as they are now, and, on the other hand, errors in the recovery of the decomposition products were induced by the enormous weight (1 cwt.) of sugar used. It may also be mentioned that Dubrunfaut (IV.), about fifty years later, employed 2559 kilos. of sugar in a single fermentation experiment. Under such conditions it required the genius of a Lavoisier, a Gay-Lussac, or a Dubrunfaut to obtain values in any way approaching the truth.

On the basis of his experiments, Gay-Lussac (II.) established the equation of fermentation as:

$$\underset{\text{Saccharose.}}{C_{12}H_{24}O_{12}} = \underset{\text{Alcohol.}}{4C_2H_6O} + \underset{\text{Carbon dioxide.}}{2CO_2}$$

(The formulæ have been rearranged in accordance with the atomic weights now accepted.) He, however, committed the error of giving an inaccurate composition of the sugar; but this was corrected by Dumas and Boullay (I.), who showed that Gay-Lussac's equation was true solely for glucose $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. As will be set forth more fully on p. 381, saccharose cannot be fermented until it has taken up a molecule of water. The products of the equation given above would necessarily amount to 48.89 per cent. of carbon dioxide and 51.11 per cent. of alcohol. Dubrunfaut (IV.) obtained in his experiment 45.17 per cent. of carbon dioxide and 46.15 per cent. of alcohol, and demonstrated that the theoretical yield of both products was unattainable.

Pasteur (XXXI.) prepared the way for more precise investigation by showing the constant appearance of by-products in alcoholic fermentation, whilst a portion of the sugar is consumed in building up the cell-substance. Elion (V.) proved that yeast assimilates sugar during fermentation, which sugar is not transformed into fermentation products. According to Wortmann (XX.) again, about 5 per cent. of the sugar present is consumed by the yeast, for the elaboration of its own substance and not for fermentation. This latter term, strictly speaking, is confined to the process of decomposition that attacks about 95 per cent. of the sugar; and therefore the full amount of the sugar cannot, in any case, undergo decomposition into alcohol and carbon dioxide.

It would occupy too much space and weary the reader to recount all the various attempts made to determine quantitatively the main products of fermentation, and we will, therefore, mention only those giving the highest results. Thus

PASTEUR (XXXI.) obtained 46.4 per cent. of carbon dioxide, JODLBAUER (I.) 46.54 per cent., and KOSUTÁNY (I.) 47.5 per cent.; whilst with regard to alcohol Pasteur obtained 48.3 per cent., Jodlbauer 48.67 per cent., and Kosutány 47.5–48.08 per cent. Hence, in the most favourable instances, the proportion of the theoretical yield amounted to 95.2 per cent. in respect of both alcohol and carbon dioxide; so that, for the bulk of the sugar decomposed during alcoholic fermentation, the equation

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O$$

—which was not directly challenged by PASTEUR (XXXI.)—still holds good.

We will also consider the case of cell-less fermentation (*see* p. 346, vol. ii.). BÜCHNER and RAPP (IX.), working with 26 grms. of saccharose, obtained 12.2 grms. of carbon dioxide and 12.4 grms. of alcohol, figures corresponding to 46.9 per cent. and 47.6 per cent. respectively. In a second experiment, however, these workers (2) obtained 50.4 per cent. of alcohol; but these figures should not be compared with those given above, the latter having been obtained with grape sugar (glucose, *see* p. 382), which furnished, theoretically, 48.89 per cent. of carbon dioxide and 51.11 per cent. of alcohol, whereas not more than 51.45 per cent. and 53.62 per cent. respectively can be formed from saccharose.

In order to obtain the above-mentioned yields highly favourable working conditions are essential; but it is immaterial whether the whole of the sugar is fermented or a portion remains behind, provided the weight of sugar actually fermented be ascertained and referred to the alcohol and carbon dioxide recovered. JODLBAUER (I). points out that, with increasing age, yeast undergoes some modification, inasmuch as the quantity of carbon dioxide produced from the fully fermented sugar diminishes progressively. This is true of pure yeast as well as of ordinary yeast. Jodlbauer obtained 49.02, 48.97, and 49.17 per cent. of carbon dioxide from saccharose, when using fresh yeast, but only 47.67, 47.44, and 46.98 per cent. with the same yeast grown old. If, on the other hand, instead of interrupting the fermentation directly after the sugar has completely disappeared, the carbon dioxide determination be carried further, a surplus of carbon dioxide, formed by the auto-fermentation (*see* § 334) of the yeast, is often obtained.

The ratio of alcohol to carbon dioxide produced is therefore approximately 1 : 1; and in zymase fermentation—according to BUCHNER and HAHN (I.)—it varies between 1 : 0.90 and 1 : 1.01. However, if the ratio be examined during the various stages of fermentation, it appears—according to LINDET and MARSAIS (I.)—that the proportion of carbon dioxide to alcohol is lower at the beginning of the process than it is toward the end. These workers obtained the following relative values of alcohol : carbon dioxide at the start :

1 : 0.93, 1 : 0.79, 1 : 0.89, 1 : 0.91, and 1 : 0.79,

and

1 : 1.01, 1 : 1.00, 1 : 1.09, 1 : 1.03, and 1 : 1.02

at the end. The results were unaffected by the fermentation temperature or by the presence or absence of acid in the wort. The amount of yeast produced per 1 grm. of alcohol was found by Lindet and Marsais to range from 0.048 grm. in the initial stage of fermentation to 0.0002 grm. at the end.

The simplest way of expressing the chemical reaction of fermentation is by the equation already given, viz.:

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O.$$

Of course the decomposition of the sugar is not effected in this elementary manner. As far back as 1858, TRAUBE (I.) expressed the opinion that the

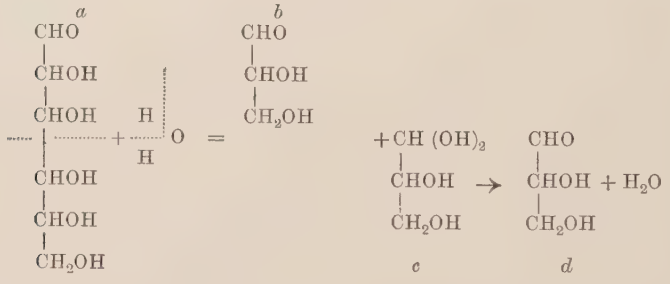
changes suffered by organic substances through the action of ferments—fermentation—are nearly always effected with the active collaboration of water. Similarly, HOPPE-SEYLER (VII.) wrote: "All fermentative processes go on in an undisturbed manner only when the solutions are in a sufficiently diluted state; and the chemical collaboration of water seems to be essential in all cases." Hence, the equation of alcoholic fermentation should be expressed as:

$$C_6H_{12}O_6 + 2H_2O = 2H_2CO_3 + 2C_2H_6O,$$

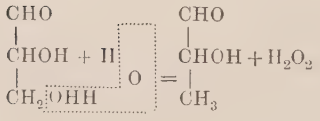
carbonic acid being formed in addition to alcohol.

A slight divergence may be permitted here. The substance generally called "carbonic acid" is really carbon dioxide or carbonic anhydride, CO_2, the acid properly so-called having the formula H_2CO_3. This latter readily decomposes into carbon dioxide and water, in accordance with the equation $H_2CO_3 = H_2O + CO_2$, so that it is merely a temporary intermediate product. According to A. BAEYER (I.) alcoholic fermentation proceeds in two stages. In the first of these, the hydroxyl groups of the sugar undergo displacement, accompanied by reduction of the one series of carbohydrate groups and an accumulation of oxygen in the other, the carbon chain then being subjected to fission where the oxygen has accumulated. This results in the formation of either the extreme anhydride of ethyl-carbonic acid or that of lactic acid—corresponding to alcoholic fermentation on the one hand and lactic fermentation on the other. Other workers, *e.g.*, WAGNER (II.), RAYMAN and KRUIS (I.), also look upon the alternate hydration and dehydration of the carbon atom as probable, without however, going more fully into the elucidation of the fermentation process.

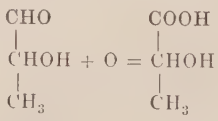
FORMULA I.



FORMULA II.

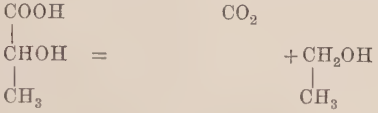


FORMULA III.



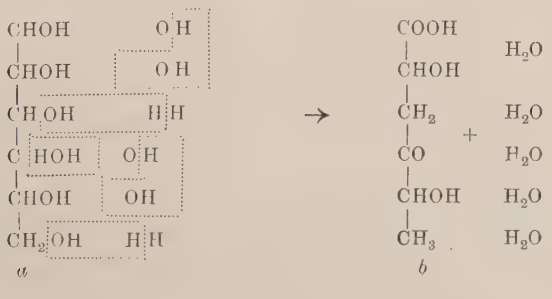
NENCKI (V.) is also of opinion that the absorption of water into the sugar molecule is essential to alcoholic fermentation, and gives an exhaustive description of the transformation of the sugar into lactic acid. We must follow his train of thought, since Buchner also has brought out a similar hypothesis with regard to alcoholic fermentation. The sugar molecule *a* (*see* Formula I.) takes up one molecule of water and decomposes into dioxypropionic aldehyde *b*, and an intermediate product *c*, which parts with water and is also transformed into dioxypropionic aldehyde *d*. This latter reacts with water (Formula II.) and

forms lactic aldehyde and hydrogen peroxide, which decomposes into water and nascent oxygen, the latter then oxidising the lactic aldehyde to lactic acid (Formula III.). Lactic acid, however, contains the elements of the chief products of fermentation, namely, alcohol and carbon dioxide:

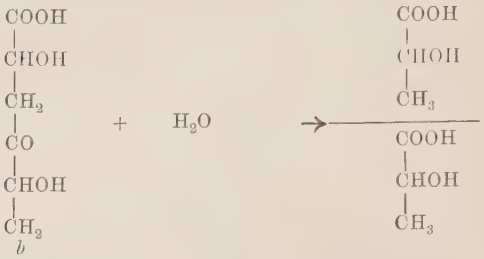


Buchner and Meisenheimer (IV.) detected acetic acid and lactic acid in their cell-less fermentation experiments (*see* p. 345, vol. ii.). The amount of this last-named acid was determined in the fresh yeast juice, the experiment being repeated after leaving the juice to stand four days with and without an addition of sugar. In two experiments without added sugar, the original lactic acid in the yeast juice was found to have disappeared, whilst with added sugar it remained, and in one case increased by 100 per cent. Of course no bacteria were present in the yeast juice. On adding 1.5 grm. of lactic acid to 500 c.c. of yeast juice and leaving for a day, the whole of the acid disappeared; but in other experiments no diminution of the added acid occurred. Subsequently, however, in three sets of experiments the formation of lactic acid was observed—whether an addition of this acid had been given or not—the amount varying, however, in inverse ratio to the quantity of acid added, owing to the adverse influence of the latter. The varying behaviour of the yeast juice samples is probably due to the character of the yeast, the tendency of the juice to produce lactic acid increasing with the age of the yeast from which it has been obtained. In cases, however, where the formation of lactic acid could be observed in the absence of added sugar, the result is obtained at the cost of the glycogen present in the yeast (*see* pp. 125, 126, vol. ii.). The chief result of this experiment is that lactic acid plays an important part in the decomposition of sugar, and probably occurs, as an intermediate product, in alcoholic fermentation. This phenomenon may be expressed graphically by the aid of Formula IV. The sugar molecule *a* (glucose) takes up four molecules of water—set down here as hydroxyl groups and hydrogen atoms—and at once parts with five molecules of water, so that a temporary intermediate product, *b*, a dioxy-γ-ketone acid, is formed. This latter combines with one molecule of water (*see* Formula V.) and decomposes into two molecules of lactic acid. That, from the theoretical point of view, this acid may be regarded as the source of the carbon dioxide and alcohol has already been mentioned on this page.

Formula IV.



FORMULA V.

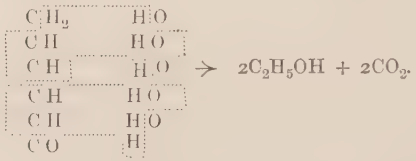


The researches of GRÜSS (I.) led him to conclude that the reducing agent he discovered in yeast (*see* chap. lxvi.) should be regarded as hydrogenase. This would first split up the sugar molecule into carbon dioxide and hydrogen, the latter then causing a separation of alcohol and water from a second and third molecule of sugar, as expressed in the following typical equations:

1. $$\left\{\begin{array}{rcl} CH_2OH + H_2O & \rightarrow & CO_2 + 5H \\ 4(CHOH + H_2O) & \rightarrow & 4(CO_2 + 4H) = 4CO_2 + 8H_2 \\ COH + H_2O & \rightarrow & CO_2 + 3H \end{array}\right.$$

2. $$\left\{\begin{array}{rcl} 2CH_2OH + H_2 & \rightarrow & C_2H_5OH + H_2O \\ 2(4CHOH) + 8H_2 & \rightarrow & 4C_2H_5OH + 4H_2O \\ 2COH + 3H_2 & \rightarrow & C_2H_5OH + H_2O \end{array}\right.$$

According to Grüss, there is a still simpler explanation of alcoholic fermentation expressed by the following formula:



This view is not open to discussion, since it coincides with the old equation $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$, which does not afford the slightest insight into the mechanism of fermentation. The other explanation put forward by Grüss presupposes the collaboration of three molecules of sugar, and is therefore more complex than the hypotheses previously mentioned. A valuable support would naturally be afforded to these latter if one could isolate the intermediate product between sugar and lactic acid, *i.e.*, Nencki's dioxypropionic aldehyde or Buchner's dioxy-γ-ketonic acid.

The remarkable property of yeast juice which brings about the disappearance of lactic acid at one time and its production at another, may be explained by the assumption of two different enzymes, one capable of decomposing sugar into lactic acid and the other transforming this acid into alcohol and carbon dioxide. With both enzymes in excess or continually replenished, only the final products of the reaction will be recoverable. This is the case in fermentation with living yeast, with which there is also the possibility of the intermediate products disappearing in consequence of the phenomena of nutrition. The first enzyme, namely, that decomposing the sugar into lactic acid, has been named zymase, or more precisely yeast zymase, by BUCHNER and MEISENHEIMER (II.), whilst they apply the name lactacidase to the second enzyme, by which the lactic acid is transformed into alcohol and carbon dioxide. These workers have

abandoned the idea that a dioxy-γ-ketonic acid is formed as an intermediate product between sugar and lactic acid; though as stated by A. Wohl and Nef, methylglyoxal may possibly be formed as a product of this kind. This is in harmony with the circumstance that inactive acid alone is invariably formed in the fermentation of saccharose and glucose, as might be expected with methylglyoxal (CH_3-CO-CHO) as the regular transition product.

The question now arises whether similar transpositions can be effected by purely chemical means, without the intervention of enzymes; and a positive result would render Buchner's hypothesis more feasible. Duclaux's (II.) observation that alcohol and carbon dioxide are formed in a solution of glucose treated with caustic potash (*see* p. 342, vol. ii.), has been confirmed by Buchner and Meisenheimer (I.), who obtained 2 per cent. of alcohol from sugar treated in this way. When caustic potash is replaced by baryta water or lime water, the carbohydrate yields 50 per cent. of lactic acid, without any production of alcohol, so that the decomposition of lactic acid into alcohol requires the action of the more powerful alkali. If potassium lactate be electrolysed by the Kolbe (I.) method, carbon dioxide and aldehyde are formed; and, according to Dossios (I.), carbon dioxide and acetic acid are produced when lactic acid is warmed with diluted chromic acid. Now, aldehyde and acetic acid are the simplest of derivatives from alcohol, so that a purely chemical proof is afforded of the presence of the atomic aggregations, carbon dioxide and alcohol, in lactic acid. According to Duclaux, calcium lactate in aqueous solution is decomposed in sunlight and air directly to alcohol, calcium carbonate and calcium acetate; and Hanriot states that the same salt furnishes large quantities of alcohol and acetone when heated with caustic lime. Mazé found that *Allescheria* (*Eurotiopsis*) *Gayoni* (*see* pp. 257 and 272, vol. ii.), also produces alcohol when grown in a nutrient solution containing lactic acid.

§ 323. The Non-volatile By-products of Alcoholic Fermentation, Glycerine, Isobutyleneglycol, Succinic Acid, Oxalic Acid, Lactic Acid.

In considering the main products of alcoholic fermentation in the preceding paragraph, it was stated that only about 95 per cent. of the total weight of the sugar is converted into alcohol and carbon dioxide.

Lavoisier (II.) had already observed the occurrence, in his fermentation experiments, of an organic acid, which he assumed to be acetic acid, corresponding to 2.63 per cent. of the fermented sugar. Pasteur (XXXI), however, was the first to emphasise the fact that alcoholic fermentation always results in by-products, the formation of which involves the consumption of about 5–6.5 per cent. of the sugar. In 1857 he named succinic acid—previously discovered by C. Schmidt (II.)—as one of these by-products, and in 1858 discovered glycerine, together with fat, cellulose and other (unnamed) substances. Following up the idea that these substances originated in the sugar, he established the following equation for the decomposition of this latter:

$$\underset{\text{Glucose.}}{49C_6H_{12}O_6} + 30H_2O = \underset{\text{Succinic acid.}}{12C_4H_6O_4} + \underset{\text{Glycerine.}}{72C_3H_8O_3} + 30CO_2.$$

From 100 parts, by weight, of sugar subjected to fermentation, Pasteur obtained 3.607–3.64 parts of glycerine, 0.673–0.76 parts of succinic acid, and 1.2–1.3 parts of other substances.

With regard to glycerine, it had already been found by Boussingault (II.) that no definite relation exists between this and the sugar consumed; and also that yeast itself furnishes glycerine when digested with water at 40°–41° C.,

30 grs. of yeast yielding 0.19–0.335 grm., or 2.5–2.87 per cent. when referred to the weight of the sugar. The production of glycerine is increased when the fermentation is accelerated by working under reduced pressure, with an augmented quantity of yeast and at a higher temperature. Both Moritz (II.) and also Thylmann and Hilger (I.) found the production of glycerine diminished by retarded fermentation and lower temperature, as well as at temperatures exceeding 35° C. accompanied by the first-named condition, whereas, on the other hand, it is increased on the yeast being provided with added nutriment, on accelerated fermentation, and by using more highly concentrated solutions of sugar. In Moritz's experiments the ratio between the alcohol and glycerine produced ranged from 100 : 9.3 to 100 : 13.8, and in those of Thylmann and Hilger, 100 : 1.638 to 100 : 11.78, *i.e.*, between very wide limits.

Moritz (II.) reports that, according to Müller-Thurgau, the amount of glycerine produced is determined by the greater or smaller vital energy of the yeast, and is in direct relation therewith, the fluctuations in the alcohol-glycerine ratio being thus explained. The absence of a definite relation between the sugar consumed and the glycerine produced was also mentioned by Straub (I.), whose results agreed in other respects with those of Kulisch (IV.) and of Thylmann and Hilger. The amount of glycerine does not increase proportionally with the alcohol content; it is increased by supplying the yeast with an abundance of food, especially such as contain nitrogenous substances.

After Müller-Thurgau, in 1884, had expressed the opinion that glycerine is a metabolic product of yeast and not one of fermentation, the following result was obtained by Wortmann (XX.) from the examination of 41 samples of must, fermented with pure yeast: namely, that the normal ratio of alcohol to glycerine varies between 100 : 7 and 100 : 14. Pure yeasts furnish a lower average yield of glycerine. The alcohol-glycerine ratio is not a criterion of the quality of the wine. The quantity of glycerine produced is not proportionate to the number of active yeast cells present, but is largely dependent on the specific glycerine-forming capacity of the race of yeast, as well as on the composition of the must. The production of glycerine is not influenced by the ash content of the must, or the quantity of yeast; and there is no mutual relation at all between the various fermentation and metabolic products. At a later date, Wortmann (VII.) expressed himself still more strongly on this point. The amount of any nutrient substance present in must or taken up by the yeast forms no measure of the quantity of any metabolic product obtained. The formation of alcohol and carbon dioxide proceeds quite independently of the formation of glycerine.

Laborde (VII.) also regards the amount of glycerine produced as a characteristic racial feature of the various yeasts, having obtained 2.5–7.75 per cent. of glycerine (mean 3 per cent.) per 100 grms. of sugar, from one and the same wine must. The production of glycerine is in inverse ratio to the activity of the yeast, and increases more particularly when nitrogenous foodstuffs to which the yeast has not been habituated—such as Liebig's meat extract, yeast water, &c.—are added to the must. An increase is also observed at higher fermentation temperatures, with stronger sugar solutions, and when the medium is strongly acidified with tartaric acid. On the other hand, a diminution is observed when artificial nutrient solutions are used, as well as in sugar solutions to which alcohol has been added previous to fermentation. To some extent the production of glycerine by a given yeast will vary according to the kind of sugar present, 3.15 grms., for instance, being obtained from galactose and inverted milk sugar, as compared with 2.45 grms. from glucose, fructose, saccharose, and maltose under identical conditions. A lactose yeast produced 1.75 grms. of glycerine per 100 grms. of sugar in a solution of lactose, but 3.16 grms. in th case of inverted lactose.

At the commencement of fermentation the formation of glycerine is smaller than toward the end, EFFRONT (XI.), for instance, obtaining at the end of

24	48	72	and 94 hours
0.15	0.35	0.40	and 0.91 per cent. of glycerine.

According to this same worker (XII.), yeast that has been habituated to preservatives (*see* vol. I, p. 192) will also produce glycerine, the capacity, however, diminishing progressively as the habituation proceeds, so that, eventually, it is possible to obtain fermentations that run their course in accordance with the theoretical equation, no by-products being formed.

A very large number of experiments have been devoted to determining the ratio between alcohol and glycerine in wine; and a few of the figures may be reproduced here. For example, according to BORNTRÄGER (XIII.), the ratio in question is 100 : 6.0; RETKOW (I.) gives it as 100 : 5.6–12.8 in white wine and 100 : 7.0–11.83 in red wine; and WINDISCH (II.) 100 : 6.1–10.2. The fluctuation of the values obtained by accurate experiment with reliable wines makes it impossible to place any legal restrictions on the amount of glycerine, and such a measure would only open the door to chicanery.

As regards the formation of glycerine in beer, BORGMANN (I.) fermented samples of one and the same wort with two different pure cultures of yeast, and found the beers to contain 0.109 and 0.137 per cent., respectively, of glycerine, the ratio of alcohol to glycerine being therefore 100 : 2.63 and 100 : 3.24. In beers prepared without pure-culture yeasts, the ratio was 100 : 4.14 to 100 : 5.497. AMTHOR (IV.) also fermented beer worts with pure cultures of eight different races of yeast, and found the percentage of glycerine remarkably low, being only 0.1113 per cent., as compared with an average of 0.144 in beer from Elsass, and 0.1266 per cent. in Bavarian beer. The minimum ratio of alcohol to glycerine was 100 : 1.65, the maximum being 100 : 4.3, and the mean 10 : 2.38.

Following the example of PASTEUR (XXXI.), most workers regarded sugar as the source of glycerine during fermentation. UDRANSKY (I.), however, who took yeast that was free from sugar and contained originally 0.053 per cent. of glycerine, and digested it with alcohol, found that, without any autodigestion having occurred in the yeast, the glycerine content increased by 116–285 per cent. of the original quantity—and even by 355.2 per cent. at the end of 13 months—though no sugar had been added. Lecithin is probably the antecedent from which the glycerine is formed, since, under certain conditions, that substance—which was also found in yeast by HOPPE-SEYLER (VII.) (*see* p. 127, vol. ii.)—decomposes into fatty acids, cholin and glycerophosphoric acid, the latter being readily split up into phosphoric acid and glycerine. DUCLAUX (XXVI. believes in the existence of special enzymes that furnish glycerine and succinic acid, pure zymase (alcoholase) probably decomposing sugar completely into carbon dioxide and alcohol. BUCHNER and RAPP (XI.) nevertheless consider that, from the chemical point of view, this decomposition of sugar is a far more complex process than the inversion of sugar, for instance, so that the constant appearance of by-products is not surprising, these being found in all complicated reactions. The problem has been solved by means of an experiment in cell-less fermentation, in which BUCHNER and RAPP (X.) found that 100 grms. of saccharose furnished 0.5 grm. of glycerine and 0.3 grm. of succinic acid, that is to say, smaller proportions than PASTEUR (XXXI.) and others obtained in fermentations with yeast. After LAXA (II.) had discovered a fat-decomposing enzyme, lipase, in yeast (*see* p. 271, vol. ii.), DELBRÜCK (XI. and XII.) expressed the opinion that glycerine is produced by the decomposition of fat (the glycerine ester of a fatty acid) by lipase. A similar hypothesis had previously been advanced by ROMMIER (II.). Distillery washes always contain fat, from the raw grain used, but in the

preparation of wort, most of the fat is left behind, though it may be assumed that a little fat—and especially lecithin—is present in the wort, in an emulsified condition if not in solution. On the other hand, the glycerine content of beer (and also wine) is so high that it can hardly be derived from the fat present in the wort. Yeast, however, according to Nägeli and Loew (III.), always contains fat (*see* p. 126, vol. ii.), small globules of which can be often detected in the cells under the microscope (*see* p. 113, vol. ii.). Fat is therefore occasionally stored up by the yeast cells, and at other times decomposed again by lipase, the fission products (glycerine) finding their way into the beer or other fermented liquid. It is probable that Buchner's expressed yeast juice contains lipase and fat (from the yeast). This supposition has much to recommend it, since, despite numerous researches, no regular connection has been discovered to exist between the amount of sugar fermented and of glycerine formed.

Isobutylene glycol was found by Henninger (I.) in a red Bordeaux wine, the amount being estimated at 0.05 per cent., or one-fiftieth of the quantity of glycerine present. It is doubtful, however, whether this substance is a fermentation product; and it most probably existed in the must already, or was formed during the process of recovery. According to Butlerow (I.), isobutylene is formed in the decomposition of amyl alcohol by heat. Sanson (I.) frequently detected isobutylene glycol, and according to Windisch (V.) it occurs also in cherry brandy.

Experiments in connection with glycerine have frequently been combined with the determination of succinic acid. Beissenhirtz (I.) is usually credited with the discovery of this substance in alcoholic fermentation, though he only found it in a case of acetous fermentation of a mixture of bread, carob beans, honey, vinegar, brandy, and water. In 1853, Schunck (II.), in the course of an alcoholic fermentation set up, as he believed, by the enzyme of madder—erythrozyme—(*see* p. 342, vol. ii.) observed the formation of carbon dioxide, a little hydrogen, and considerable quantities of alcohol, accompanied by a small amount of succinic acid. However, the proof that this acid is a constant by-product of alcoholic fermentation was due to C. Schmidt (II.) and Pasteur (XXXI.), the latter, as already mentioned, obtaining 0.673 0.76 per cent. of this acid from sugar, and explaining its formation by the equation given on p. 365, vol. ii.

Broadly speaking, the hypotheses on the formation of succinic acid are the same as in respect of glycerine. According to Boussingault (II.), the yield of succinic acid increases with the temperature, quantity of yeast, and reduction of atmospheric pressure; Norin and Cloudon (I.), on the other hand, stating that it decreases when air is excluded, and increases when the fermentation is conducted with access of air. The amount produced during the various stages of fermentation varies. Kayser and Dienert (I.) found that the quantity increases at first, diminishing toward the close of fermentation, whilst Effront (XII.), on the contrary, observed a continuous increase in the amount of this acid, the maximum being reached in the final stages of the process, *e.g.*:

after 24	48	72	and 96 hours
0.025	0.045	0.068	and 0.092 per cent.

Thylmann and Hilger (I.), *inter alia*, ascribe the increase or decrease of succinic acid to the same causes that operate in the case of glycerine.

Exhaustive researches on succinic acid were undertaken by A. Rau (II.), who, for the most part, employed 15 per cent. solutions of saccharose, glucose, and maltose, with or without nutrient substances. Three different yeasts were employed, at temperatures of 15°, 25°, and 35° C., air being excluded in some cases; and the principle of intermittent fermentations also applied. The results

showed that the total acidity is considerably increased at the higher temperatures, no alteration in this respect being obtained by the addition of nutrient substances. No great fluctuation was observed in the content of succinic acid, nor does the yield appear to be seriously influenced by the kind of sugar, or the presence or absence of air or of yeast foods. Pure yeast and pressed yeast, with vigorous fermentative power, gave a higher yield of succinic acid than ordinary beer yeast. The formation of the acid goes on, *pari passu*, with the production of alcohol and decomposition of the sugar. With intermittent fermentation, and at 35° C., however, the ratio of alcohol to succinic acid was 100 : 0.439; but in the three subsequent pauses the ratio was 100 : 0.875, 100 : 0.89, and 100 : 0.823 respectively, or practically identical. A comparison of the production of glycerine and succinic acid shows that low temperature, whilst unaffecting the formation of the acid, restricts that of glycerine. The presence or absence of nutrient substances has no influence on the production of the acid, whereas providing the yeast with abundant nutrition causes an increase in the yield of glycerine. (This excessive feeding of the yeast probably leads to an accumulation of fat, which is afterwards decomposed—*see* pp. 367, 368, vol. ii.). The production of succinic acid is independent of that of glycerine, and Pasteur's equation (*see* p. 365, vol. ii.) is inapplicable. This view of the formation of succinic acid is also shared by Straub (I.). Duclaux (XXVI.) attributes it (like glycerine) to the action of a separate enzyme, but adduces no proof in support of the hypothesis.

Succinic acid is produced in fermentation with expressed yeast juice. In one case, reported by Buchner and Rapp (X.), 1250 c.c. of the juice contained 0.2 grm. of the acid before fermentation, but after the fermentation of 100 grms. of saccharose, the amount was found to be 0.5 grm., an increase of 0.3 grm.

Opinions are still divided with regard to the source of succinic acid, most workers regarding sugar as the raw material—as in the case of glycerine—though it is worthy of note that the amount of this acid produced under different conditions of fermentation is invariably small. The amount of succinic acid present in beer is also small, *i.e.*, 0.0026–0.0039 per cent. according to Straub (I.). Blumenthal (I.) states that micro-organisms produce this acid from both carbohydrates and protein, on which account Grüss (II.) believes that the source, in the latter case, is to be found in asparagin, the assumption being that this substance is first transformed by yeast oxydase into aspartic acid, which is then decomposed into malic acid, according to the equation:

$$2\,\begin{matrix}CH - CO(NH)\\ |\\ CH(NH_2) - COOH\end{matrix} + 3O_2 = 2\,\begin{matrix}CH - COOH\\ |\\ CH(OH) - COOH\end{matrix} + 2N_2 + 2H_2O$$

Asparagin — Malic acid

Owing to the simultaneous presence of a reducing substance (*see* chap. lxvi.) in the yeast cell, the abstraction of the hydroxyl group from the malic acid might be effected by its agency, succinic acid, $COOH\text{-}CH_2\text{-}CH_2\text{-}COOH$, being thus formed. According to the Grüss hypothesis, free nitrogen must be formed; but this has not yet been observed during alcoholic fermentation. With regard to the quantitative proportions in which this element could appear in comparison with carbon dioxide, 1 grm. of sugar could—taking Pasteur's figures as a basis—yield 464 mgrms. of carbon dioxide and 7.6 mgrms. of succinic acid, the formation of the latter being accompanied—according to the Grüss equation—by the liberation of 1.8 mgrm. of nitrogen. Converted into volume, these figures would be equal, at 15° C. and 760 mm. pressure, to 253 c.c. of carbon dioxide and 1.5 c.c. of nitrogen, or, in round numbers, 0.6 per cent. of the gas.

Pasteur (XXXII.), who determined the carbon dioxide volumetrically, does not mention the presence of nitrogen; but he himself described the experiment as a very delicate one, so that the smaller amount of nitrogen that might be produced with a diminished yield of succinic acid might well escape detection. In order to substantiate the Grüss hypothesis it would be necessary to test the fermentation gases for the presence of free nitrogen, and to show that this latter is formed in direct ratio to the amount of succinic acid produced. It would also have to be proved that yeast actually forms succinic acid from asparagin.

Oxalic acid is formed during fermentation by various organisms, *e.g.*, by *Saccharomyces Hansenii*, Zopf (XV.)—compare p. 209, vol. ii.—but it has not been definitely found to result from alcoholic fermentation by yeast, though crystals of oxalate (*see* p. 86, vol. ii.) are often observed when yeast is examined under the microscope, their presence being ascribed by Prior (V.) to the formation of small quantities of oxalic acid during fermentation. Whether this originates in the sugar, however, or was already formed in the fermented solution, is quite undecided.

Lactic acid was discovered in certain fermentations by Dubrunfaut (IV.) in 1856, though it should be remembered that he did not work with pure yeast, and, since lactic bacteria are abundant, experiments of this kind, to be worthy of consideration, must be performed with yeast perfectly free from bacteria. Pasteur (XXXI.) was unable to detect any lactic acid in the fermentations with *his* pure yeast; and only in one single instance did he mention that a very small quantity of the sugar had been converted into lactic acid. Effront (XII.) ascribes the formation of lactic acid to a transformation of the proteid substances. In the fermentation of sugar by expressed yeast juice, Ahrens (I.) observed a non-volatile acid, which he stated to be lactic acid, but Buchner and Meisenheimer (IV.) were the first to prove in an indubitable manner that this acid is a constant by-product of alcoholic fermentation, and originates in the sugar (*see* p. 346, vol. ii.).

§ 324. Volatile Acids and Aldehydes as By-products of Alcoholic Fermentation. Influence of Oxygen on Fermentation.

The acids in § 323 are classified as non-volatile, or fixed acids, and are contrasted, in zymotechnology, with the volatile acids, all of which—so far as concerns those present in fermentation products—belong to the fatty series. Prior (VI.) fermented one and the same beer wort with seventeen different pure yeasts, and determined the acidity in the resulting beers. The quantity of the fixed organic acids formed per 100 c.c. of beer ranged from the equivalent of 2.1 to 5.4 c.c. of decinormal caustic soda, and that of the volatile acids between 2.1 and 5.8 c.c. of alkali. For every 100 c.c. of alkali required to neutralise the fixed organic acids, the quantity consumed in neutralising the volatile acids formed by the various yeasts was 62.4–180.9 c.c. Consequently the acidity due to the several races of yeasts fluctuates between wide limits. According to Alfred Rau (II.), the quantity of volatile acids formed increases considerably at higher fermentation temperatures (35° C.); and pressed yeast produces a larger amount than beer yeast. Straub (I.) found that admission of air increases the production of volatile acids; and according to Biourge (I.), the quantity formed is independent of the alcohol produced. The concentration of the fermenting liquid is without any appreciable influence, but, on the other hand, the yield is proportional to the duration of fermentation, especially if the completely fermented solution be stored for some time. Amthor (II.) found this to be especially the case with *Saccharomyces apiculatus* (*see* p. 323, vol. ii.).

Formic acid is the first member of the volatile series. It was observed here as long ago as 1891, by KRUIS and RAYMAN (II.), in beers that had been left standing in contact with deposited yeast for some years (*see* p. 92, vol. ii.). They also found it in sterile beer worts after prolonged storage, and therefore attributed its formation to a chemical reaction occurring in the wort itself, and probably connected with the transformation of protein. KHOUDABABACHIAN (I.) detected formic acid in fresh grape must, the quantity increasing during fermentation when the surrounding conditions were unfavourable to the yeast. According to LIEBERMANN (IV.) and KITICSAN (I.), traces also occur in normal wines, though, as observed by DUCLAUX (I.), it disappears readily in presence of yeast. THOMAS (II.) mentions the occurrence of formic acid in aqueous infusions of malt culms; nevertheless it is also formed in fermentation, especially when a large surface of the liquid is exposed and nitrogen compounds are present. Of these latter, urea, either alone or in association with ammonium bicarbonate, is best adapted to increase the output of formic acid, which can be still further augmented by the addition of calcium carbonate. During cell less fermentation, BUCHNER and MEISENHEIMER (I.) found traces of a volatile acid very similar to formic acid. The frequent occurrence of the latter among the products of bacterial fermentation has already been mentioned on p. 140 of vol. i.

Acetic acid was recognised at an early date as a by-product of alcoholic fermentation, by LAVOISIER (II.). DUCLAUX (XXVII.) and BÉCHAMP (XIII.) also found acetic acid constantly, though only as traces; and these observations were confirmed by SCHÜTZENBERGER (II.). Owing to the widespread occurrence of acetic bacteria, the acid can only be regarded as a by-product of alcoholic fermentation when yeast, free from bacteria, is employed; just as was remarked with regard to lactic acid. KRUIS and RAYMAN (II.) failed to detect acetic acid at all, though they made it the object of special attention; whereas THOMAS (II.) obtained it regularly, though merely as traces. BUCHNER and MEISENHEIMER (IV. and II.) found it invariably in fermentations with expressed yeast juice free from bacteria; and it is therefore certain that the acid is a normal by-product of alcoholic fermentation. The yeast juice, prior to fermentation, contained 0.004–0.010 per cent. of acetic acid, and afterwards 0.08–0.33 per cent., so that considerable quantities were produced during the operation. The specimens of yeast juice that fermented lactic acid furnished considerable quantities of acetic acid, the converse being the case with such of them as produced lactic acid in abundance. The name glucacetase has been applied to the yeast enzyme that is assumed to split up glucose into three molecules of acetic acid. The hypothesis advanced by BIOURGE (I.) to account for the formation of acetic acid is that the volatile acids must be regarded as the result of assimilation processes, and not of the decomposition of sugar, yeast itself yielding a considerable amount of acid on distillation. According to PRIOR (V.), however, it is more feasible to suppose—and this is indicated by the experiments of GILTAY and ABERSON (II.)—that the oxygen consumed in fermentation oxidises a portion of the alcohol in the interior of the yeast cell. The influence of oxygen on fermentation—a point to which we shall revert shortly—also plays a part in this case. It cannot, however, be credited as the sole cause of the production of acetic acid during fermentation, the experiments of BUCHNER and MEISENHEIMER (IV.) having proved this acid to be a normal by-product of that phenomenon. It is true that the amount so produced is small, though, according to THYLMANN and HILGER (I.), it is greater when the solution contains a higher proportion (30–40 per cent.) of sugar. When it is formed to any extent otherwise, its production should perhaps be attributed to the oxidation of the alcohol, probably with the assistance of bacteria. Acetic acid, though only in small quantities, is also found in fusel oils.

The third member of the fatty-acid series is propionic acid, which was found by WINKLER (II.) and BÉCHAMP (XIV.) in diseased wines. According to ORDONNEAU (I.), it is also occasionally present, as an ester, in cognac. STRECKER (I.) and KRAMER (I.) attribute its origin to lactic acid, in the case of certain fermentations—a point worthy of note, seeing that this acid is a normal by-product of alcoholic fermentation.

Butyric acid, which is readily produced by certain bacteria, was detected—either in the free state, or more frequently combined as an ester—in cognac by DUCLAUX (XXVIII.) and ORDONNEAU (I.), and in potato fusel oil and cherry brandy by K. WINDISCH (III. and IV.).

Valeric acid was found by KRUIS and RAYMAN (II.) in a sterilised wort that had been kept for several years. They ascribed its origin—as well as that of the other higher fatty acids still to be mentioned—to the decomposition of complex nitrogenous foodstuffs, *e.g.*, protein. DUCLAUX (XXVIII.) has also found it occasionally in diseased wines.

Among the higher fatty acids, caproic acid, caprylic acid, pelargonic acid and capric acid occur, in combination with alcohols as esters, in the fusel oils (*see* next paragraph) and in cognac, whilst œnanthylic acid appears only in wine, or cognac. On this point *see* PELOUZE and LIEBIG (I.), DELFFS (I.), FEHLING (I.), A. FISCHER (I.), GRIMM (I.), DUCLAUX (XXVIII.), ORDONNEAU (I.), K. WINDISCH (III. and IV.), KRUIS and RAYMAN (II.), and SCHÜPPHAUS (I.).

Taking the total fatty acids as representing 100, WINDISCH (III.) gives the proportions of the individual fatty acids in the esters as follow: in potato fusel oil 3.5 of acetic acid, 0.5 of butyric acid, 14 of caproic acid, 34 of caprylic acid, 12 of pelargonic acid, and 36 of capric acid; in corn fusel oil, acetic acid 2.7, butyric acid 0.4, caproic acid 13.2, caprylic acid 26.7, pelargonic acid 12.9, and capric acid 44.1. HILGER (I.) also found stearic acid, palmitic acid and lauric acid in a sample of corn spirit. These acids originate undoubtedly from decomposed fat; and it is highly probable that the other higher fatty acids, from butyric acid to capric acid, are produced from the fat (*see* pp. 367, 368, vol. ii.) contained in the mash and in the yeast cells, as the result of decomposition by yeast lipase; compare BAU (XXII.).

The aldehydes that are always formed during fermentation should be regarded as intermediate products between the fatty acids and the alcohols. Ordinary aldehyde (acetaldehyde) was observed by BÉCHAMP (XIII.) and RÖSER (II.). The cause of its formation will be dealt with shortly. As was shown by DURIN (II.), it can be easily recovered by strongly cooling the fermentation gases in suitable vessels. In works where yeast is produced by the aeration process, a considerable amount of aldehyde is formed under the influence of atmospheric oxygen, especially in mashes of maize, rice, and malt, the resulting spirit having an evil smell and taste in consequence of its high content of aldehyde—*see* MÆRCKER (II.). JAKSCH (III.) also states that aldehyde is formed during alcoholic fermentation. KAYSER (XIII.) regards aldehyde as a product of the activity of the *Saccharomycetes;* and, according to KRUIS and RAYMAN (II.), considerable quantities of this substance are formed when a film is produced and there is a plentiful accession of air. Hence aldehyde is the result of the oxidation of nascent alcohol. According to Ilges, it is not formed during fermentation, but only in the distilling apparatus, by contact of the spirit vapour with air—compare MÆRCKER (IV.). During the oxidation of alcohol, the formation of aldehyde is accompanied by the production of acetal, the diethyl ether of aldehyde, $CH_3.CH(OC_2H_5)_2$; and, according to GEUTHER (I.) and WINDISCH (IV.), it occurs in fairly considerable quantities in fermentation products, whilst ORDONNEAU (I.) found it in cognac. Its formation can be easily explained, since, according to DURIN (II.), alcohol and aldehyde come into

contact in the nascent state during the formation of the latter substance, so that the two may unite to acetal, with elimination of one molecule of water.

The influence of oxygen on alcoholic fermentation by yeast will now be considered, though its action on cell reproduction and respiration has already been dealt with exhaustively on p. 169 *et seq.*, vol. i. It may be mentioned at once that the favourable influence of aeration on the fermentation of must, worts, and mashes has long been recognised in practice, and was fully established by a series of fermentation technologists between the years 1867 and 1874. Blankenhorn did this in an imperfect manner, then Moritz (partly in collaboration with Haas); also by Molnar in the case of wine musts, and by Adolf Mayer (VII.) with nutrient solutions. More accurate researches were undertaken with wort by R. Pedersen (I.) in 1878. It was ascertained that aeration increases both the working action on the extract and also the (absolute) reproductive power of the yeast, though the amount of extract consumed per unit weight of the yeast crop is smaller in aerated worts than in others. The difference, however, is not large—as was proved arithmetically by D. Iwanowski (I.) in 1893. In repeating Pedersen's experiment, E. C. Hansen (p. 170, vol. ii.) found that the amount of extract consumed per cell of the yeast is smaller in cultures that have been aerated. The results of this test, however, cannot be accepted unconditionally, the passage of air being accompanied by subsidiary effects, inasmuch as it sets up vibrations in the wort liquid and thus stimulates reproduction. According to Rapp (I.), the fermentative activity on the other hand is diminished by powerful vibration, so that the aforesaid stimulative effect is counteracted to an extent that has not been precisely determined. Moreover, the air in its passage carries off volatile metabolic products, including those of an injurious character, thus freeing the nutrient medium from poisonous constituents, to an extent varying according to circumstances. This favourable influence is absent in all the parallel experiments in which a similar "rousing" with inert gas has not been performed. If hydrogen (which is very difficult to obtain in a perfectly pure state) be used for this purpose, it is stated by Korff (I.) that the resulting acids differ considerably from those formed when in aerated cultures, a circumstance that will readily be understood in view of the chemical action exerted by atmospheric oxygen.

The difficulty and complication of the task of investigating the influence of oxygen on alcoholic fermentation by means of yeast are increased by the powerful stimulus imparted by this gas to the reproduction of the cells. An attempt to counteract this disturbing factor was made by A. J. Brown (IV.). Starting from his own observation that no appreciable reproduction occurs in wort pitched with a larger number of cells than can be grown therein from a minimum sowing, he made large sowings in a mixture of yeast water and glucose, passing either air, hydrogen, or carbon dioxide through the liquid. By operating in this way it was found that, whilst the number of cells remained practically unaltered throughout the experiment, the amount of sugar fermented was larger in the case of the aerated cultures. The conclusion deduced therefrom, that a given number of cells of approximately the same total weight will ferment more sugar in presence of air than without, was opposed by Duclaux (XVIII.) on arithmetical grounds, which, however, were rejected by Brown (VII.).

Brown's experiments need to be repeated, as was pointed out by H. van Laer (XI.) and Iwanowski (I.), since the assumption on which they are based conflicts with general experience. The absence of any reproduction in the excessive sowing was probably due to some unfavourable constitution of the nutrient medium employed, a factor whose influence has been shown by the experiments of N. von Chudiakow (I.), who supports the view that oxygen has

a restrictive influence on alcoholic fermentation. Another circumstance left entirely out of consideration is the fact that a number of cells perish during fermentation and undergo dissolution, so that when the same quantity of yeast is found at the beginning and end of the process, it cannot be assumed, with certainty, that no reproduction has occurred. An unduly large sowing of yeast also modifies the composition of the nutrient medium, owing to the diffusion of soluble matters from the yeast, more particularly from the dead cells. It is still an open question, however, whether this change persists when air, carbon dioxide, or hydrogen that is not perfectly pure, is blown through the solution.

Although the results of the majority of these experiments tend to indicate that the amount of work performed per unit of yeast is smaller in presence of oxygen, and that oxygen therefore restricts fermentation, they cannot, however, be regarded as decisive. For this to be the case it is an essential condition to show that each cell has been continuously exposed to the influence of the oxygen throughout the entire experiment. Now, in the case of liquid cultures, the yeast cells frequently agglomerate to small lumps, the interior of which cannot be reliably demonstrated to be accessible to oxygen; and the metabolism proceeding inside these lumps differs from that in the outer cells that are exposed to the ascending bubbles of air. This objection applies with still greater force to all cultures on solid media, including streak cultures on sugar-gelatin, the cells below the surface being practically shut off from the oxygen in contact with those on the surface. The only way to afford decisive proof is by experiments in cell-less fermentation with zymase, and therefore the question whether the fermentation set up by yeast is influenced in one way or another by oxygen must be regarded as still unsettled.

Practical experience in the alcohol industry is not opposed to the foregoing particulars. The purpose of the aeration regarded as necessary or useful in the case of fermenting mashes, especially molasses, distillery wash and pressed yeast factories, is mainly to increase the yeast crop and not to augment the fermentative power of the individual cells. Any prolongation of the rousing process beyond the attainment of this object is for the purpose of utilising the favourable supplementary effects of this treatment as indicated on p. 373, vol. ii. Due precaution must be observed in this, since otherwise considerable loss may arise from another supplementary effect, namely, the volatilisation of the alcohol. These circumstances are merely referred to now, to prevent the impression that the fact that aeration accelerates and increases the fermentation of a liquid medium affords proof that oxygen stimulates fermentation. The amount of alcohol carried away by the liberated carbon dioxide was investigated by Eck (I.) as long ago as 1875, in the case of distillery washes; and the loss determined by Riss (I.) in experiments made with a saccharine medium containing mineral salts, amounted to 1.12 per cent., referred to the quantity of alcohol present in the fermented liquid.

Finally, brief consideration may be devoted to Pasteur's conception of alcoholic fermentation as life without air (vol. i. p. 16). The experimental basis on which this theory was constructed, so far as yeast is concerned, was shown to be untenable by Nägeli (II.). The assumption that the plentiful admission of oxygen to cultures of yeast causes this latter to develop like an aerobic thread fungus and not set up alcoholic fermentation has not been proved, such proof entailing the determination of the ratio between the carbon dioxide liberated, the alcohol produced, and the resulting yeast crop. The solution of this task was first undertaken by Giltay and Aberson (II.); but their experiments are open to objection, though not in the direction mentioned by Duclaux (XVIII.) in a criticism refuted by Giltay (I.). This worker and Aberson found that over 60 per cent. of the sugar consumed was converted into alcohol and carbon

dioxide, even in strongly aerated yeast cultures in which energetic respiration occurred at the same time. A similar result was obtained by Buchner and Rapp (V.); but neither set of experiments was conclusive, the conclusions being only a matter of probability, and therefore tending to refute the accuracy of Pasteur's hypothesis.

The chemical action of oxygen in alcoholic fermentation is a far simpler question than this physiological influence. As already stated on pp. 371, 372, vol. ii., alcohol readily undergoes oxidation to aldehyde and acetic acid. According to Durin (II.), the carbon dioxide liberated during fermentation constitutes a compact froth, and the alcohol distributed all over the surface of the minute bubbles of gas is readily transformed into aldehyde. Röser (II.) also found a larger quantity of aldehyde in fermentation conducted with admission of air than without aeration. In any event, however, some action is exerted by yeast oxydases in connection with the carrying of oxygen, especially when a film is produced by the yeast. If a fermented liquid be left, together with the whole of the yeast, in contact with air for some time, Kruis and Rayman (II.) state that nearly all the alcohol formed during fermentation is oxidised to carbon dioxide and water. The part played by atmospheric oxygen during the storage of wines was referred to by Pasteur (XXXIII.), who found that young wine will retain its original character for a long time when stored out of contact with air. Which of the substances, however, combine with the oxygen, and what products result, still remain undetermined. According to Wortmann (VII.) there is no doubt that considerable changes are produced by atmospheric oxygen during the storage of wine (*see* p. 379, vol. ii.); but the view that has prevailed since Pasteur's time, namely, that the matter is one of oxidation, is not applicable in its entirety since physiological processes probably form a contributory factor. The changes in question are chiefly ascribed by Wortmann to the collaboration of organisms. According to Rapp (I.), the formation of esters is also increased by the access of air—a circumstance that can be easily explained, since the acids resulting from the oxidation of alcohols combine more readily with alcohol, in the nascent state, to form esters, than is the case with acids already formed.

§ 325. Alcohols and Esters (Bouquet Principles) as Volatile By-products of Alcoholic Fermentation. Other By-products.

Whereas, in the preceding paragraphs, we have dealt with the volatile products belonging to the aliphatic series and containing relatively large amounts of oxygen and little hydrogen—such as the volatile acids and the aldehydes, with the general formulæ, $C_nH_{2n}O_2$ and $C_nH_{2n}O$—we will now proceed to treat of bodies with the formula $C_nH_{2n+2}O$, the first of which series is methyl alcohol, $CH_3.OH$. This is often formed during bacterial fermentation, but may also occur through the decomposition of intermediate products (glucosides) in alcoholic fermentation by yeast. This circumstance will be referred to later on (*see* p. 380, vol. ii.).

The second member of the series, namely, ethyl alcohol, one of the chief products of fermentation, has been dealt with in § 322.

The higher alcohols are abundantly represented in cognac, and in the fusel oils discovered by Scheele (II.) in 1785. They occur in the free state, and also as esters in combination with fatty acids.

Primary propyl alcohol, $CH_3.CH_2.CH_2.OH$, was obtained by Chancel (I.) from the fusel oil of grape-husk spirit, and also by Fittig (I.). According to K. Windisch (III. and IX.), Kruis and Rayman (II.) and Ordonneau (I.), it occurs constantly in crude potato and corn spirit, cherry brandy and cognac.

Secondary, or isopropyl alcohol, $CH_3.CH(OH).CH_3$, is said to have been obtained by Berthelot (VIII.), and was found in crude potato spirit by Rabuteau (II.).

Normal butyl alcohol, $CH_3.(CH_2)_2.CH_2.(OH)$, is formed, according to Fitz (XIII.) and Emmerling (VII.), in the fermentation of glycerine (a constant by-product of alcoholic fermentation) by fission fungi. It was isolated from crude potato spirit by Rabuteau (II.), from crude corn spirit by Emmerling (VII.), and from cognac by Ordonneau (II.). According to this last worker, it is probably a normal product of fermentation by wine yeasts, whereas beer yeast, which furnishes other secondary products, produces isobutyl alcohol, $(CH_3)_2.CH.CH_2.CH_2(OH)$. This last substance had already been obtained by Wurtz (I.) from the crude spirits furnished by beetroot, potatoes, and grain; it was also found by Pierre and Puchot (I.), K. Windisch (III. and IV.), Rabuteau (II.), and by Kruis and Rayman (II.).

Primary pentyl or amyl alcohol, $CH_3.(CH_2)_3.CH_2(OH)$, seems, according to Wyschnegradsky (I.), to occur along with the isomeric amyl alcohols in fusel oil.

Fermentation amyl alcohol, or isoamyl alcohol, constitutes the bulk of fusel oil. It occurs as ordinary, optically inactive alcohol, $(CH_3)_2.CH.CH_2.CH_2(OH)$, but, according to Marckwald (I.), is invariably accompanied by the active alcohol

$$\begin{matrix} CH_3 \\ CH_3.CH_2 \end{matrix}\!\!>\!CH.CH_2(OH)$$

which rotates the plane of polarised light towards the left. Le Bel states that it is converted into the dextro-rotatory modification under the influence of mould fungi. The name, amyl alcohol, was bestowed by Cahours (I.) because the alcohol is formed from materials containing starch. The amyl alcohols are found in all technical fusel oils, and to a smaller extent in cognac as well; compare also Pedler (I.), Krutsch (I. and II.), Balard (I.), and Halenke and Kurtz (I.).

Various hypotheses have been advanced to account for the formation of the fusel oils. According to Brefeld (XVIII.), by-products are formed as soon as the materials requisite for the continued growth of the yeast are exhausted, the yeast dying and decomposition setting in as fermentation progresses. If the higher alcohols originate in normal fermentation, the amount so formed must, *pace* Lindet (IV.), bear a constant relation to the quantity of ethyl alcohol during the various stages. This, however, is not the case, the amount of fusel oils formed during the first fourteen hours being 0.36 part per 100 of alcohol, but 14.07 parts in twenty-four hours. This increase is apparently due to micro-organisms which do not come into action to their full extent until fermentation has terminated. The higher alcohols are products of a secondary fermentation, and are also formed, to a smaller extent, when the fermentation is accelerated by increasing the quantity of pitching yeast, or by adding sterile beer wort.

Perdrix (I.) established the fact that bacteria are able to produce fusel oils, this worker having isolated from Seine water a bacterium that furnished amyl alcohol. Péreire and Guignard (I.) found a similar bacillus in calcareous waters, and thought of utilising it technically; and Pringsheim (I.) described an amylic bacillus, isolated from American potatoes. Perdrix's bacillus, however, produces such a small quantity of this alcohol that it cannot be regarded as the sole agent in the formation of fusel oils. The experiments of Lindet (IV.), which showed that the amount of higher alcohols formed remained nearly constant, despite modifications in the conditions, might be explained by bacterial activity, although, as a matter of fact, it is not quite clear why the action of the bacteria was not influenced by the modifications in question. Stronger proof of

bacterial agency in this connection is afforded by the experiments of Gayon and Dupetit (I.). The quantity of the fusel oil formed can be considerably lessened by the addition of bactericidal substances or by powerful aeration, which suppresses anaerobic bacteria. It should be borne in mind that in nearly every case, except the so-called "Amylo process" (*see* pp. 69 *et seq.*, vol. ii.), the grape, potato, and grain mashes employed for fermentation are only imperfectly sterilised (addition of malt!), if at all, so that bacteria *may* gain access from the start.

In the case of brewing, where the worts are mostly sterilised, few reports are met with, in the literature, concerning the formation of higher alcohols during fermentation. Chapman (I.) distilled six samples of English beers and found, per 100 parts of crude alcohol, 0.051–0.250 part of fusel oil, as amyl alcohol, 0.021–0.062 part of esters, chiefly ethyl acetate, and traces of furfural. As mentioned already on p. 376, vol. ii., Ordonneau (I.) ascribes the formation of the higher alcohols to the vital activity of the yeast itself. Rayman and Kruis (I.) attempted to decide the question by fermenting sterile worts with pure cultures of four different yeasts and one of a species of *Mycoderma*. All the experiments in which *Sacch. cerevisiæ*, L., furnished a low yield of fusel oil, were performed with cells that had been cultivated for a long time in the laboratory under unfavourable conditions. It seems that the formation of amyl alcohol results from the yeast having reached a certain state of exhaustion. According to Mærcker (V.), on the other hand, it should be pointed out that grain mashes, which are particularly well adapted for the energetic nutrition and reproduction of yeast, greatly favour the production of fusel oil.

A high fermentation temperature is said by Kruis and Rayman (II.) to increase the yield of fusel oils. A yeast, otherwise incapable of producing fusel oil in malt worts, furnished a large quantity of amyl alcohol in an imperfectly sterilised yeast mash that had turned sour spontaneously with formation of lactic acid; but no higher alcohols were formed by yeast and aeration in a yeast that had been completely sterilised after turning sour. It was also found that in all cases where fusel oil was produced, the amount of acetaldehyde formed was merely small, and *vice versâ*. Hence, anaerobiotic conditions seem to contribute essentially to the formation of the amyl alcohols.

According to Gentil (I.), the above experiments do not sufficiently prove that fusel oils are produced by yeast, owing to the conditions adopted, namely, the selection of a yeast previously inhabiting a medium containing amyl alcohol, and the weakening of the yeast by an abnormally high temperature and shortened fermentation. Gentil himself failed to obtain amyl alcohol in fermenting a solution of saccharose containing malt peptone as yeast food.

In subsequent experiments, Kruis and Rayman (III.) found that, contrary to the results previously obtained, the formation of amyl alcohol is unaffected either by unfavourable composition of the nutrient medium or by the age and physiological condition of the yeast. The alcohol is formed only when certain carbohydrates are present; and the assumption is put forward that amyl alcohol is formed, not from the hexoses (§ 326), but from other sugars that result from the polymerisation of the polysaccharides always present in the cereals employed as the raw material. In ten experiments with glucose, fructose, saccharose, and beet juice, ethyl alcohol alone was produced, though, on the other hand, amyl alcohol in considerable quantity resulted from the use of barley worts and inverted brewers' grains, which substances contain fat (*see* next page).

Emmerling (XI.), on the other hand, believes, from his tentative experiments, that fusel oils are not produced in more than minimum quantities, if at all, in fermentations from which bacteria have been rigorously excluded. He

concludes that the amyl alcohols originate in carbohydrates, and are produced by bacteria that are of widespread occurrence and are almost invariably found on the skin of potatoes.

Great influence on the formation of fusel oils is exerted by the nature of the substance to be fermented, the presence of fat being a contributory factor according to Bornträger (II.). Fermentations with materials that have been freed from fat yield very little fusel oil.

Sugar and carbohydrates are usually regarded as the sources of the higher alcohols; but, according to Bau (XXII.), there is another possibility that should not be left out of consideration, namely, the formation of these substances from the fat that is ready formed in the mash and is generated from sugar by yeast, to be stored up and afterwards decomposed again. During fermentation the process of hydration is accompanied by those of oxidation and reduction. Moreover, in industrial mashes, one has to reckon with the presence of bacteria, a number of which are endowed with powerful reducing properties. According to Durin (III.), aldehydes are formed, not only by the oxidation of alcohol, but also by reducing actions occurring during fermentation, the nascent aldehyde being then capable of easy reduction to alcohol.

Lactic acid (*see* p. 363, vol. ii.) may be regarded as the originating material for the formation of propyl alcohol, this alcohol being found, according to Bouchardat (I.), among the products of lactic fermentation. Fitz (XIV.) states that it is also formed during the fermentation of glycerine by fission fungi.

According to Bau (XXII.), the higher alcohols originate from the fatty acids derived from fats; and indeed, butyric acid, caproic acid, caprylic acid, and capric acid are frequently met with in fats. When these acids are liberated by lipase (*see* p. 367, vol. ii.), they may, in the nascent state, be reduced to alcohols, especially in symbiotic fermentations by yeast and bacteria; indeed, numerous organisms are known that even eliminate free hydrogen.

There is one difficulty in the way of explaining the formation of the amyl alcohols by this hypothesis. They must be assumed to originate in valerianic acid, which, according to Kruis and Rayman (II.) is formed, with other higher fatty acids, from nitrogenous compounds of complex structure. Even though, *pace* Brieger (I.), valerianic acid be actually capable of formation from protein, it is hardly feasible to suppose that this alone forms the source of the large quantities of amyl alcohol found in the fusel oils. Cahours and Demarçais (I.), it is true, state that fats yield valerianic acid by chemical means on distillation with superheated steam. Nevertheless, according to the highly important researches of Ehrlich (I.), both the amyl alcohols are formed from leucin and isoleucin (the fission products of protein), under the influence of the normal vital activity of yeast. D-leucin forms the source of the levo-rotatory *d*-amyl alcohol, whilst *r*-leucin is split up so as to form isoamyl alcohol and *d*-leucin. About 87 per cent. of the *l*-leucin is transformed by yeast into amyl alcohol. According to Effront (XIV.), the autodigestion of yeast (*see* chap. lxvi.) forms another source of amyl alcohol, which, however, does not begin to appear until the process has reached an advanced stage. As the yeast cells die off, the formation of amyl alcohol ceases—a proof that this alcohol is produced, not by the vital activity of the yeast cells *per se*, but by the action of an enzyme excreted by the living cells. Hence, to a certain extent, Effront's opinion is at variance with the results of Ehrlich's experiments and consequently further investigation is required concerning the formation of fusel oil.

Methylpropylcarbinol, $C_3H_7CH(OH)CH_3$, was discovered by Rabuteau (I.), and hexyl alcohol, namely, primary isohexyl, or caproyl alcohol, $(CH_3)_2C_4H_7(OH)$, was detected, by Faget (I.), in the fusel oil of grape-husk spirit. According

to K. Windisch (III.), this alcohol occurs, in small quantity, in crude grain spirit; and it has also been found by Kruis and Rayman (II.) in crude potato spirit.

The presence of small quantities of heptyl alcohol or œnanthyl alcohol, $C_7H_{15}.OH$, was confirmed by K. Windisch (III.) in crude grain spirit, and Faget (II.) obtained it from grape-husk spirit. Probably the alcohol recovered in the latter case was a primary isoheptyl alcohol, $(CH_3)_2C_5H_9(OH)$.

Finally, both normal and secondary nonyl alcohol, $C_9H_{19}.OH$, were discovered in crude potato spirit by Hilger (I.).

According to Rommier (II.) the fatty acids and alcohols produced during fermentation frequently combine while in the nascent state to form esters, which are also formed during the prolonged storage of fermented worts (wine) or distilled spirits (cognac). These bodies constitute a large proportion of the "bouquet" principles. The esters identified include the acetic acid compounds of ethyl and amyl alcohol, and the corresponding compounds of butyric acid and the other higher fatty acids. The distillation of wine yeasts furnishes, according to Pelouze and Liebig (I.), œnanthic ether, which later investigations have shown to consist chiefly of ethyl caprinate. In addition to traces of other esters, it is also said, by Delffs (I.), to contain ethyl pelargonate, and—by A. Fischer (I.)—caprinates and caprylates. According to Liebig, 40,000 parts of wine furnish 1 part of œnanthic ether, to which the characteristic odour of wine is principally due.

Wortmann (XVI.) and Mastbaum (I.) assert that the bouquet principles may be divided into four classes: (1) those originating in the raw materials; (2) those produced during the saccharification of the mash and during fermentation; (3) those formed during storage; and (4) those generated by distillation. The discussion of the first group does not come within the scope of the present chapter, although the substances concerned possess special importance in connection with the bouquet of wine and the character of many beers (hops). The odorous substances formed during fermentation are the above-mentioned esters of the alcohols and fatty acids, all of which are volatile. Some of these substances, the cause of the bouquet of wine, seem to be still unidentified, chemically. Some yeasts, notably Lindner's (XLII.) fruity ether yeasts, produce large quantities of esters, chiefly acetic ether. In addition to these volatile bouquet principles—with which should be classed the fruity-smelling neutral ethyl succinate—certain non-volatile esters occur in wine, and probably also in beer. According to K. Windisch (V.) these esters contribute largely to the flavour of wine. They include acid ethyl succinate, and the fermentation esters of tartaric acid and malic acid, both of which acids exist, ready formed, in the grape. In addition to the primary bouquet principles—in the sense adopted by Kosutany (II.) and Wortmann (XVI.)—introduced by the grapes themselves, various odorous substances are produced by the different yeasts. On this point compare Kosutany (II.), Mach and Portele (III.), and Pichi (II.).

The bouquet principles developed during the storage and ripening of wine appear to result principally from the influence of oxygen (*see* p. 375, vol. ii.). Chouard (I.) found that a bouquet principle formed during primary fermentation; disappeared afterwards, probably as a result of some reducing process in fermentation; the bouquet may, however, reappear during storage, in consequence of oxidation. According to Wortmann (VII.), the processes involved in this case are physiological, and not merely chemical reactions.

Changes, apart from continued fermentation, also occur during the storage of beer; these, according to Nathan (II.), relating chiefly to the elimination of immature bouquet principles which impart an unripe flavour to the beer.

The distilled spirits, cognac and brandy, also undergo changes in storage,

which changes are influenced by esterification, as well as by other factors, such as storing the products in casks that are (in contrast to those used for beer) neither lined with pitch nor varnished.

Besides these true by-products of alcoholic fermentation, we have to consider substances that exist ready formed in the raw materials, and are decomposed under the influence of fermentation. These substances are principally glucosides which, on being decomposed by enzymes, yield up their components to the fermented liquid. This explains the occurrence of hydrocyanic acid, benzoic acid, benzaldehyde, and benzaldehyde-cyanhydrin, for instance, in cherry brandy —according to K. Windisch (IV.)—and also of methyl alcohol in fermented fruit juices. In these latter, prepared from plums, cherries, and apples, Wolff (I.) found, almost invariably, methyl alcohol in the proportion of 1 per cent. of the ethyl alcohol present. In the case of wines prepared from grapes with the stalks unremoved, the resulting alcohol contained about 0.15–0.4 per cent. of methyl alcohol, whereas wines from grapes freed from stalks furnished 0.03 per cent. at most. The alcohol obtained by fermenting sugar with wine yeast was, on the other hand, entirely free from methyl alcohol in every case.

The nitrogenous constituents of the yeast (*see* p. 159, vol. ii.) and of the raw material undergo changes during fermentation. Thus fermentation products have been found to contain the following volatile and non-volatile compounds: ammonia, by Kruis and Rayman (II.) and by K. Windisch (IV.); trimethylamine, and other amines, by Ordonneau (I.) and Ludwig (I.); pyridin, collidin, &c., by Kramer and Pinner (I.) and Ordonneau (I.); *b*-glycosin, by Morin (I.) and Tanret (V.); derivatives of pyrazin and other bases, by Schrötter (I.), Oser (I.), Guérin (I.), Stöhr (I.), E. Bamberger and Einhorn (I.); leucin and tyrosin.

The distillates of fermented mashes and liquors also contain other chemical compounds, which were formerly believed to originate, at least in part, during alcoholic fermentation. Foremost among these is furfural, which was found by Kruis and Rayman, more particularly associated with the formation of large quantities of acetaldehyde. Furfural was discovered in 1882 by K. Förster (I.), in crude spirit, and also in the distillates from wine and beer. He ascribed its formation to the effect of the heat (boiling temperature) on the pentosans (*see* pp. 150, 151, and 181, vol. ii.) contained in the raw materials. Kruis and Rayman regard furfural as a product of the metabolism of yeast, a view opposed by Chapman (I.). According to Lindet (VI.), it is formed only during the fermentation of worts from raw materials (cereal grains) that have been dissociated with acids, or when the fermented mash has been distilled by direct fire heat. No furfural is obtained when the starch has been saccharified by diastase, and distillation has been effected by steam; so that it is not a product of fermentation. According to W. Windisch (IV.), it is produced by boiling acid solutions of carbohydrates, especially the widespread pentoses and pentosans, and is therefore formed in the distillation of the invariably slightly acid mashes, wines, and beers. This explains why C. Heim (I.) found Munich beer to be destitute of furfural. Brand (II.) and Heim (I.) failed to obtain confirmation of W. Windisch's (II.) hypothesis that the pasteurisation flavour of beer is due to furfural. It is, however, certain that when beer containing no furfural is boiled for a sufficient time, furfural makes its appearance; and the same is naturally the case with distillery mashes and wine. The test recommended by Lenz (I.), namely, that the occurrence of the furfural affords decisive proof that a sample of cognac is a pure wine distillate, is unreliable.

K. Windisch (III. and IV.) states that fermentation products have also been found to contain terpene and terpene hydrate, as well as oils of high boiling-point, derived from the raw materials. Under certain conditions, sulphur compounds

may also occur in the products of industrial fermentations, K. Windisch (V.), for instance, having found sulphuretted hydrogen in wine, whilst Barbet (I.) and Elwart (I.) observed sulphurous esters in spirits produced from molasses and sulphured saccharine juices. The formation of these compounds may be readily explained by the reducing action of yeast enzymes in presence of free sulphur or sulphur dioxide.

§ 326. Sugars Susceptible of Direct Fermentation.

As already mentioned on p. 360, vol. ii., saccharose will not ferment until it has taken up a molecule of water, in which operation it is transformed by the enzyme, invertase (*see* § 327), into two hexoses, glucose and fructose, according to the equation:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

For a long time this observation was unique of its kind, all attempts made to convert maltose—which has the same empirical composition as saccharose—into two hexoses by a similar enzymatic decomposition, having failed; so that it was held that maltose undergoes direct fermentation (compare Morris (III.), Hansen (LXIV.), Dastre (I.), Dünnenberger (I.), Mehring (I.), and Donath (II.)). E. Fischer (VII.), however, established beyond dispute that maltose is split up into two molecules of glucose by a special enzyme, maltase (*see* § 328), as a preliminary to fermentation. His discovery (VIII.), in collaboration with P. Lindner, that saccharose is also hydrolysed by *Monilia candida* (*see* p. 331, vol. ii.), which does not contain ordinary yeast invertase—which discovery was followed by that of other instances—raised to the status of fact the axiom that all the di- and poly-saccharides must, in order to be capable of alcoholic fermentation by yeast, be first split up into simpler sugars by special yeast enzymes (which will be described later on). This circumstance forms the basis of differentiation between the directly fermentable sugars and the di- and poly-saccharides. The former are compounds, the carbon atoms of which are arranged in a simple chain, whilst the others are ether-like substances, in which separate carbon chains are connected together by one or more oxygen atoms. Those desirous of going more thoroughly into the study of the sugar group may be referred to the works of Tollens (II.) and E. O. von Lippmann (II.).

For the fermentable simple and compound sugars, E. Fischer (IX.) established the axiom that only such as contain a number of carbon atoms divisible by 3 are susceptible of true alcoholic fermentation by yeast.

The first members of this group would be the trioses ($C_3H_6O_3$), which do not occur in nature, but have been prepared artificially and play an important part in the synthesis of sugars. According to E. Fischer (X.), alditriose or *i*-glycerose is capable of alcoholic fermentation; but this is contested by Wohl (I.) and Emmerling (VII.). This sugar is readily condensed to a compound with the formula $C_6H_{12}O_6$, which sugar is the cause of fermentation phenomena in glycerose syrup. The same applies to ketotriose (dioxyacetone), which is represented by the constitutional formula $OH.CH_2.CO.CH_2.OH$, and was obtained by Bertrand (VII.) in the fermentation of glycerine with *Bact. xylinum*. According to Emmerling (VIII.), it is unfermentable, the slight fermentation phenomena that make their appearance after prolonged warming being attributable to the condensation of the triose into a hexose.

Mention may also be made here of *d*-manno-nonose, a sugar that is also unknown as a natural product. It has the formula $C_9H_{18}O_9$ and, according to E. Fischer (XI.), is readily and completely fermented. Another sugar to be borne in mind is *d*-glycoheptose, which Lindner (XLII.) succeeded in fermenting

by means of a yeast (No. 691 of the collection at the Berlin Brewing Institute) from the mucinous secretion of oak-trees. Since this sugar contains 7 atoms of carbon—in accordance with the formula $CH_2OH.(CHOH)_5.COH$—this observation urgently needs confirmation, since if it be correct it disposes of Fischer's axiom respecting the triplicity of the carbon atoms of fermentable sugars.

At one time it was also thought that the pentoses, *i.e.*, sugars with the formula $C_5H_{10}O_5$, including xylose and arabinose, as well as rhamnose, a methylpentose $C_6H_{12}O_5$, were susceptible of true alcoholic fermentation by yeast. A prolonged controversy was maintained on this point, on account of two circumstances: first, the mixed fermentations caused by the use of impure sowings of yeast, and secondly (where pure yeast was used), the neglect to consider the fact that yeast is also able to assimilate such sugars and utilise them in the construction of new cells which are unable to ferment them. Lactose forms a well-known example of this kind. It seems by no means impossible that, given a sufficiently large sowing of yeast and small amount of sugar, the whole or a portion of the sugar present may be eliminated without any true fermentation taking place. Of course, where impure yeast or unsterilised nutrient solutions are used, the action of bacteria may come into play, a number of which are known to be capable of producing alcohol from pentoses. The proof that alcoholic fermentation cannot be set up in the pentoses by yeasts has been given by a number of workers, including Tollens and Glaubitz (I.), Scheibler, Cross and Bevan (I.), Stone and Tollens (I.), Smith, E. O. von Lipmann (III.), E. Fischer (XVI.), and P. Lindner (XXXV.). According to Buchner and Rapp (III.), expressed yeast juice is also inactive toward pentoses.

The real directly fermentable sugars are the hexoses, which have the general formula $C_6H_{12}O_6$; and indeed, only such members of this group as belong to the *d*-series, the *l*-compounds being unfermentable. Here also, as in the other kinds of sugars mentioned, a distinction is drawn between the aldoses and ketoses.

The most widely occurring and best known of the aldo-hexose sugars is *d*-glucose, also known as dextrose, grape sugar, starch sugar, and diabetic sugar. It is fermented by all organisms capable of inciting alcoholic fermentation, and therefore by all culture yeasts—of which, according to Lange (IV.), about 700 races are already known—and all wine yeasts. (*See also* pp. 150 *et seq.*, and p. 296, vol. ii.)

The sugar, *d*-mannose, also known as isomannose, seminose and carubinose, is only occasionally met with in nature, for example—according to Tsukamto (I.)—in the Japanese *Amorphophallus Konjaku*, and according to Prinsen-Geerligs (V.), Pellett (I.) and others, in various kinds of colonial molasses, in orange rind; and also, according to Gruss (III.), temporarily in germinating dates. On the other hand, it forms a regular constituent of the mannanes, which are of widespread occurrence throughout the vegetable kingdom and represent, to some extent, condensation products of mannose, either by itself or in association with other sugars. In the latter case the products are classed as conjugate mannanes. This sugar is fermented by all Lindner's yeasts (XXXV.) which also ferment *d*-glucose, except *Saccharomyces membranafacieus*, *S. farinosus*, *S. Bailii*, a *S. apiculatus* from Leipzig mead and one from raspberry juice, *S. exiguus*, *Endoblasterma amycoides I.*, *E. liquefaciens*, a film yeast from marshmallow sap, a fruit-ether yeast from gall fermentation, and *Schizosaccharomyces Pombe*.

Whether *d*-galactose—also formerly termed lactose (not to be confounded with the di-saccharide lactose, or milk sugar, of the existing nomenclature) and lactoglycose—actually occurs in a free state in nature has not yet been definitely ascertained. In combination with *d*-glucose it forms milk sugar and melibiose. In the vegetable kingdom, *d*-galactose is found as a constituent of several

glucosides, and especially of the widespread galactans, which may be divided into simple and conjugate galactans. True galactans are met with in barley, malt, and numerous seeds, and, according to PRINSEN-GEERLIGS (V.) and E. O. von LIPMANN (III.), also in the products and waste products of the cane-sugar and beet-sugar industries. According to PAYEN (III.) and BAUER (II.), gelose, the chief constituent of agar-agar, consists mainly of galactan. Conjugate galactans occur in vegetable mucilages: in yeast gum according to SCHÜTZENBERGER (III.), as galactoarabans in various seeds, according to E. SCHULZE (V.), whilst LINTNER (VIII.) states that galactoxylan is a constituent of wheat, barley and malt. Conjugate galactans, differing with the origin of the material, are found in gum arabic. These bodies are also met with in the animal kingdom: associated with milk sugar in milk, according to BÉCHAMP (XV.); whilst according to THUDICHUM (I.) they form a constituent of protagon. LINDNER (XLII.) states that *d*-galactose is fermented by all yeasts that dissociate *d*-glucose, except the following species; *Sacch. membranæfaciens*, *S. farinosus*, *S. Bailii*, *S. apiculatus*, *Schizos. Pombe*, *Schizos. mellacei*, as well as a few yeasts from gall fermentation and cucumber pickle. On the other hand, strangely enough, it is fermented by two film-producing budding fungi (Nos. 127 and 374 of the Berlin collection) which leave glucose, mannose, and fructose intact. This report urgently needs confirmation, since the older statements on the fermentability of *d*-galactose are more divergent than in the case of any other sugar. It would occupy too much space to detail the communications on this point, and the reader is therefore referred to BAU'S work (XXIII.), in which the older literature was critically reviewed. According to E. Fischer, galactose is fermented by the culture yeasts, by *S. pastorianus I. II.* and *III.*, *S. ellipsoideus I.* and *II.*, and *S. Marxianus*, as well as by milk-sugar yeast, whereas no fermentation is set up by *S. membranæfaciens* and *S. productivus*. KOZAI (II.) reports that saké yeast will also ferment galactose.

Whilst the above three sugars of the hexose group are aldoses, the following representative of the ketohexoses must be added: *d*-fructose (levulose or fruit sugar), which is very widespread in nature and almost invariably accompanies *d*-glucose. A mixture of these two sugars in equal parts constitutes invert sugar, which term is also applied to their mixtures in any proportion. Fruit sugar is a constituent of several polysaccharides, including saccharose, melitriose, lupeose, stachyose, &c., of inulin and allied substances, and of yeast lævulan (*see* p. 128, vol. ii.). It is fermented by all organisms capable of fermenting *d*-glucose, with the single exception of a film yeast, LINDNER'S No. 178 (XLII.).

Only the above four hexoses (*d*-glucose, *d*-mannose, *d*-galactose and *d*-fructose) are fermented by yeasts, all the others being unfermentable.

CHAPTER LXV.

ENZYMES DECOMPOSING DISACCHARIDES AND POLYSACCHARIDES.

By Dr. A. BAU.

§ 327. Invertase.

The best known and most frequently investigated yeast enzyme is invertase, which was originally termed invertin, and has also been called saccharase, sucrase and euinvertase.

In its occurrence it is one of the most widespread of the enzymes. In the animal organism it is found in numerous organs, especially in the mucous membrane of the small intestine (particularly in warm-blooded animals): and it has also been found, by Erlenmeyer and A. von Planta (I.), Axenfeld (I.) and others, in insects. In the vegetable kingdom it occurs in the majority of plant organs: leaves, flowers and fruit (also in hop cones), &c., though in far smaller amount than in the lower fungi, mould fungi, yeasts and bacteria.

Invertases of different origin are not always of identical composition; and differences of action on saccharose are also observed in the invertases according to the method of preparation employed—compare Fernbach (VI.). For this reason, Bau (XII.) proposed the name "euinvertase" for the invertase occurring in the true yeast, until further investigation had shown whether the saccharose-dissociating enzymes obtained from such highly divergent materials were really identical. A special example of the variation in the individual invertases is afforded by *Monilia candida* (p. 331, vol. ii.) which, though decomposing and fermenting saccharose, does not, in the opinion of Fischer and P. Lindner (II.), contain true invertase. Hahn (III.) regards the enzyme of this fungus as an endoenzyme which is possibly combined with the protoplasm; and perhaps it is merely an enzymogen. Buchner and Meisenheimer (I.), who investigated the expressed juice of *Monilia candida*, found that the enzyme will not diffuse through parchment paper, differing remarkably, in this respect, from yeast invertase.

Invertase occurs extensively in yeasts, being found in all culture yeasts used in brewing, distillery work and pressed yeast making, and therefore in all top- and bottom-fermentation yeasts of the *Sacch. cerevisiæ* type, both of the Frohberg and Saaz races comprised in the types OF, OS, UF, and US (*see* p. 402, vol. ii.). All the true wine yeasts also contain invertase; but, on the other hand, it is absent from *Schizos. octosporus* (*see* pp. 202–208, vol. ii.). Invertase is, however, present in a number of other budding fungi, though not in any of the examined races of *Sacch. apiculatus* (*see* p. 321, vol. ii.) and most *Torulaceæ* (*see* p. 296, vol. ii.). Of the latter, however, Hartmann's (I.) *Torula colliculosa* ferments saccharose without difficulty, whereas other species are incapable of decomposing this sugar. In this connection, further investigation is urgently required, since, in most of the work already done, attention was mainly directed to ascertaining whether the organisms employed were able to ferment saccharose, and not to determining the presence of invertase.

In the preparation of invertase in the purest, *i.e.*, most active, condition possible, it is the almost universal practice to employ *Sacch. cerevisiæ*, of either top- or bottom-fermentation type. In the older methods the first stage was to kill the yeast by means of alcohol or ether, in order to extract the invertase afterwards with water or glycerine—compare Berthelot (III.), Liebig (III.), Hoppe-Seyler (IX.), Gunning (I.), and Donath (III.). The resulting solution is precipitated, fractionally, with strong or absolute alcohol, under which treatment the earlier fractions have less enzymatic power than the later ones. The precipitates are washed with absolute alcohol, and dried in the desiccator. In other methods, according to Barth (II.) and Amthor (V.), the yeast is first carefully warmed, to expel the bulk of its moisture, and is then dried more energetically, and the resulting powder is extracted. An exceedingly powerful solution of invertase is obtained by allowing yeast to ferment spontaneously—O'Sullivan and Tompson (I.)—or by recovering the expressed juice by the Buchner method. According to Issaew (I.), plasmolysing pressed yeast with saccharose will furnish a very active invertase, from which the dissolved saccharose can be eliminated by fermentation. The relatively purest invertase, however, is obtained by killing the yeast and extracting it with glycerine or water, whether the killing be effected by treatment with alcohol or ether, or, preferably, by heating the carefully dried yeast to 100° C. and over. According to Osborne (I.), the yeast, after being killed with alcohol, should be digested with chloroform water at a moderate heat for some time, the filtrate being poured out into 96 per cent. alcohol. The deposited flakes are washed with alcohol, dried, and dissolved in 25 parts of water, the earthy phosphates still present being thrown down by a careful addition of ammonia, and the filtrate dialysed and then evaporated *in vacuo*. Wroblewski (IV.) also employed dialysis for purifying the enzyme; but this worker precipitated the invertase beforehand by saturating its solution with ammonium sulphate.

The chief property of invertase is its power of hydrolysing saccharose, which it splits up into one molecule each of *d*-glucose and *d*-fructose according to the equation

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

Whether the enzyme is also capable of hydrolysing other sugars is doubtful: *see* § 332 on this point.

As already mentioned on p. 381, vol. ii., yeast cannot ferment saccharose directly, the sugar needing to be first hydrolysed by invertase. It is uncertain whether this decomposition is effected inside or outside of the yeast cell. According to O'Sullivan (II.), the healthy yeast cell is incapable of diffusing invertase; and consequently the hydrolysis of the saccharose within the cell must precede fermentation. This opinion is shared by Hiepe (I.), who considers that hydrolysis is intimately connected with cell protoplasm, and that the operation is one in which physiological laws play as important a part as chemical laws; furthermore, that the admission of the saccharose into the cell, and the outward passage of the products of inversion are physiological processes. According to Fernbach (VI.), the rate at which yeast cells permit the escape of the enzyme is in inverse ratio to their age. In this connection Pottevin and Napias (I.) examined five yeasts in a peptonised solution of saccharose, and found that four of the races ceded invertase to the medium in the early stages of fermentation, whilst the fifth did not. These four yeasts yielded powerful solutions of invertase when macerated with chloroform water; but the fifth only parted with a little invertase after digestion for a fortnight. Hence the individual races of yeast appear to differ in respect of the cession of the enzyme to the circumambient medium. In general, however, it may be assumed that fresh

yeast cells belonging to the groups *Sacch. cerevisiæ* and *Sacch. ellipsoideus I.* will allow invertase to diffuse through their cell membrane. Bau (XXIV.) and Donath (IV.) nevertheless found invertase in all fermented beverages.

On the constant occurrence of this enzyme in beer, Bau (XXV.) established a method of detecting whether beer has been pasteurised. One 20 c.c. sample of beer is boiled, and a similar quantity is left unboiled, each being treated with 20 c.c. of a 20 per cent. solution of saccharose, then kept for twenty-four hours at room temperature, treated with 0.5 c.c. of lead acetate, made up to 50 c.c. with distilled water, filtered and polarised. Should an appreciable difference be observed in the deviation of the angle of polarisation in the polarimeter, the beer has not been pasteurised; but if the two results be identical, or approximately so (slight differences in the reading being due to experimental error), the beer will certainly have been pasteurised, and probably at a temperature exceeding 57° C. In the absence of a polarimeter, the test may be performed as follows: 5 c.c. of Fehling solution are boiled with 1 c.c. of test liquid (40 c.c. of beer and 40 c.c. of saccharose solution, after digestion for twenty-four hours). If the liquid remain blue, with a slight red precipitate, no invertase is present; in the opposite event the Fehling solution will be reduced completely.

Enzymatic action is also greatly influenced by temperature in the case of invertase; and whilst this action begins at about zero C., the optimum temperature is considerably higher. In the case of invertase from top-fermentation pressed yeast, A. Mayer (XI.) found this optimum temperature at 31° to over 36° C., and 44°–48° C. in that from bottom-fermentation yeast. On the other hand, according to Kjeldahl (I.), the optimum temperature for the activity of invertase from bottom yeast is 52.5° C., and that for the enzyme from top yeast, 56° C. A. Mayer (XI.) considers that these divergencies are explained, on the one hand by the invertase preparations being injured in the course of production, *e.g.*, by treatment with alcohol, and, on the other hand, by the fact that adherent impurities have a stimulating or restrictive influence on the enzymatic action according to their character. After very careful investigation O'Sullivan and Tompson (I.) determined the optimum temperature at 55–60° C. A considerable difference also exists in the reports as to the temperature at which this enzyme is destroyed, the explanation being the same as just given. For instance, alcohol lowers the destruction temperature, whereas high concentration and the presence of glycerine has the opposite effect. Prolonged exposure to a constant temperature also has an injurious effect, A. Mayer (XII.), for instance, finding the enzyme to be destroyed at 51° C. in some cases, whereas in others it remained active, though weak, at 65° and even at 66° C. According to O'Sullivan and Tompson (I.), the destruction temperature of invertase is 75° C.; and the same result was obtained by Bau (XXVI.), who did not prepare the enzyme in a pure state, but examined it direct in the cell by the Bokorny (IV.) method. This method obviates the injury always suffered by the enzyme in the course of isolation; but, on the other hand, allowance must be made for the fact that, when the experiment is repeated, the yeast may not be in the same physiological condition in all the tests. It is true that the conditions of nutrition of the yeast do not modify the properties of the enzyme; but its quantity and activity may be influenced by the accumulation or diminution of other substances present in the yeast cells. From additional experiments made, it may be assumed that yeast invertase, provided it has remained uninjured, will develop its maximum activity at 52°–56° C., and that it is certainly destroyed in aqueous solutions, and also in the yeast cell, by a temperature of 75° C. In an absolutely dry state it will stand far higher temperatures. Both pure invertase and dry yeast will stand heating, without loss of enzymatic power, to temperatures assessed by A. Mayer (XIII.) at 97° C., by Bau (XII.)

at 100° C., by Buchner (III.) at 145° C., and by Salkowski (X.) at as high as 160° C. According to Bau (XXVI.), yeast that has been dried at the ordinary temperature or heated to 105° C. retains invertase even at the end of five and three-quarter years.

In investigating the influence of chemical reagents on invertase, Bokorny (IV.) followed the principle of allowing these reagents to act on the yeast itself, in order to obviate any injury that the invertase may suffer in preparation. He reports that invertase remains unimpaired when the yeast is stored in absolute alcohol for three days at ordinary temperature, or for twenty days in 50–75 per cent. alcohol. The enzyme is also uninjured when the yeast is kept for two days in solutions containing 0.25–0.60 per cent. of oxalic acid, 0.1–0.5 per cent. of hydrofluoric acid, 2 per cent. of acetic acid, 2 per cent. of lactic acid or 5 per cent. of formaldehyde. The enzymatic power is also not destroyed by small quantities of mineral acids, alkalis, arsenites, hydrocyanic acid, chloroform, phenols, toluene and thymene, both of which latter were employed by Emil Fischer and P. Lindner (II.) in their investigations on enzymes. Similarly, Bau (XXVI.) examined yeast by digestion at 12°–17° C. for twenty-nine hours, and found that the invertase was destroyed by treating the yeast with 1 per cent. and 0.5 per cent. sodium hydroxide, and 0.1 per cent. silver nitrate, a weakening effect being produced in the case of 0.1 per cent. mercury chloride, whereas solutions of lower concentration remained inert. No injury was suffered by the invertase on treatment with organic acids, including tartaric acid of 4 per cent. strength.

With regard to the influence of light on invertase, the reports of workers differ. A. Mayer (XIV.) and Emmerling (XII.) failed to discover any such influence; but according to Downes and Blunt (I. and II.) and also Duclaux (XXIX.) the enzyme is sensitive towards light, especially in presence of air. Very dilute acids stimulate the activity of invertase; but the quantity used must be smaller in the case of mineral acids than of organic acids. For instance, according to Fernbach (VII.), 0.0025 per cent. of sulphuric acid in the solution produces optimum activity, whereas the same result requires the presence of 1 per cent. of acetic acid. Moreover, the reports of various workers differ on this point, *e.g.*, those of Kjeldahl (I.), Dumas (VII.), Nasse (I.), Loew (X.), O'Sullivan and Tompson (I.) and Fernbach (VI.); presumably because they worked with invertase of divergent origin and method of preparation, and containing different extraneous substances. According to Nasse (II.), carbon dioxide accelerates hydrolysis by this enzyme, whereas carbon monoxide and oxygen have the opposite effect. All alkalis and alkaline salts are said by Duclaux (XXIX.), O'Sullivan and Tompson (I.), and Fernbach (VI.) to have strongly adverse influence, even in small quantities. According to Nasse (II.) and Duclaux (XXIX.), small quantities of alkali chlorides and calcium chloride have a beneficial effect, whilst salts of the heavy metals are injurious. Alcohol, even as little as 5–10 per cent., is stated by A. Mayer (XV.), J. Moritz (III.), and O'Sullivan and Tompson (I.) to have a restrictive influence on hydrolysis; and, according to Griffith (I.), small quantities of salicylic acid have a similar effect.

In contrast to other enzymes, invertase seems to be completely inalterable. A. Mayer (XVI.) found that it is not attacked by putrefactive bacteria, although his experiment was not entirely free from objection, it being stated by Fermi and Montesano (I.) that certain bacteria themselves produce invertase, so that there is no proof whether the invertase found in the products of putrefaction really originated in the yeast or were excreted by the bacteria. Bau (XXVI.) investigated the mutual interaction of yeast enzymes, of which yeast-endotryptase (*see* chap. lxvi.), or yeast-peptase alone come under consideration. Yeast that had been liquefied at 45° C., or expressed yeast juice that had been kept for

one to three weeks at 17°–20° C., or heated at 30° C. or 40° C. for an hour, still contained unimpaired invertase. The activity of this enzyme also remained intact when the yeast was digested for twenty-four hours at 37° C. with a solution containing the extremely large quantity of 1 per cent. of pepsin (Merck) and 0.1 per cent. of hydrochloric acid. It is true that, in these experiments, nothing was done to ascertain the quantities of invertase before and after the treatment with yeast endotryptase and pepsin respectively.

Attempts have already been made at the quantitative determination of invertase; but the method proposed by Fernbach (VIII.), like all quantitative methods for the determination of enzymes, is attended by the drawback that only the effect of the enzyme can be measured and not the amount of enzyme actually present. According to Fernbach, a number of samples (each measuring exactly 4 c.c.) of a 50 per cent. solution of saccharose are treated with 1, 2, 3, &c., c.c. of the invertase solution under examination, each of the mixtures being then treated with 1 c.c. of decinormal acetic acid and made up to 10 c.c. The test-glasses are then warmed to 56° C. for an hour on the water-bath, cooled quickly and treated with a few drops of caustic soda to destroy the enzymatic action, the amount of invert sugar formed being determined by means of Fehling's solution. Fernbach estimates the unit of invertase as that capable of hydrolysing 0.2 grm. of saccharose in one hour at 56° C. and in presence of 1 per cent. of acetic acid.

According to Moritz and Morris (I.), the hydrolysis of saccharose by invertase is utilised in certain English breweries by digesting beer yeast with the saccharose solution at 56° C. and running the inverted mixture into the hop back.

Invertase is also utilised practically in chemical analysis, for the determination of saccharose in cases where no reliable results can be obtained either by direct polarisation or by the Clerget-Herzfeld inversion method. According to the Convention for the Uniform Examination of Foodstuffs and Delicacies, Vereinbarungen (I.), 100 c.c. of the solution under examination, *e.g.*, a 10 per cent. solution of honey, are treated with 50 c.c. of a solution of invertase, prepared by the conventional method, the mixture being allowed to stand for two hours at 50°–55° C., and the invert sugar then determined either in the polarimeter or gravimetrically.

§ 328.—Maltase.

Whereas at one time it was thought that maltose was capable of direct fermentation, we have already seen, on p. 381, vol. ii., that this sugar also must be subjected to hydrolytic fission before it can be attacked by alcoholase.

Maltose, which was first discovered by Dubrunfaut, is also known as malt sugar, and, in the anhydrous condition, has the same empirical composition as saccharose, namely, $C_{12}H_{22}O_{11}$. Unlike the latter, however, it is not composed of two "simple" sugars, but consists of two molecules of *d*-glucose, condensed to maltose by the elimination of water. It occurs in nature, usually in small quantities in the leaves of various plants and, according to Puriewitsch (VIII.), is formed during the germination of seeds. It has also been found in germinated barley, and occasionally in green and cured malt, by numerous workers, including O'Sullivan, Brown and Morris (III.), Jalowetz (II.), and others, whereas other observers, such as Düll (III.), Lintner (IX.), and Kröbler (I.) deny or regard as doubtful its presence in malt. These divergent results are explained to some extent by the circumstance that some workers extract the malt with water, in order to examine the sugar content, during which treatment the diastase is afforded an opportunity of acting on the starch, whilst others have attempted to destroy the diastase previous to extraction for the practical

purpose of the fermentation industry; however, it is immaterial whether maltose is already contained in malt or not, since the mashing process in brewing and distilling is designed for securing a more or less extensive conversion into maltose of the starch contained in the cereal grains.

In distillery work and in the manufacture of pressed yeast, attention is concentrated on attaining the utmost possible saccharification of the starch by diastase, whereas in brewing it is found desirable to regulate the process of saccharification, according to the type of beer required, in such a manner, by the employment of more or less highly cured malt, that the wort will contain a larger or smaller quantity of maltose, according as the beer is to be lightly fermented and full flavoured, or highly fermented and vinous. In this connection it is customary to speak of the "attenuation" (degree of fermentation) of the beer, which, however, does not depend solely on the mashing process, but also on the kind of pitching yeast (*see* p. 197, vol. ii.) employed.

Though maltose can be hydrolysed by acids, the transformation—which results in the production of two molecules of *d*-glucose—is far less easily effected than is the case with saccharose. On the other hand, maltose is readily decomposed by the yeast enzyme maltase. A similar enzyme was discovered in maize by Geduld (II.), who termed it "glucase"; but later workers, including Lintner and Kröber (I.) have shown them to be different.

Maltase was discovered in yeast by Emil Fischer (VII.), after Lintner (X.) had indicated the possibility of yeast possessing an enzyme capable of decomposing maltose. The original name for the enzyme was glucase or glycase, it being also called yeast glucase for closer identification; but at that time the nomenclature of the enzymes was in a state of confusion, some of them being named after the products to which they give rise, and others after the sugars they decompose (compare W. Windisch (V.)) and it was only later that the term maltase found general acceptance for the enzyme that decomposes maltose. In order to obviate any uncertainty, E. O. von Lippmann (IV.) proposed a new terminology, according to which the enzymes were to receive double names, the first portion indicating the sugar decomposed, and the second the product, or main product of the hydrolysis. Under this proposal the enzyme decomposing maltose would be termed maltoglycase or maltoglucase; but this name has not come into favour.

Maltase occurs in all races of culture yeasts of the *Sacch. cerevisiæ* group belonging to the UF, US, OF, and OS types, as well as all wine yeasts (compare pp. 205, 206, vol. ii., and p. 209, vol. ii.). Special interest attaches to Hartmann's (I.) *Torula colliculosa* (*see* p. 295, vol. ii.). As already mentioned, maltase also occurs in maize; likewise in mould fungi (*see* p. 268, vol. ii.), turnips, peas and potatoes, as well as in cereals (compare Beijerinck (XIII.), and Stoklasa and Czerny (I.)). The low enzymatic influence exerted on maltose by barley leads to the supposition that, as in the case of wheat, rye, and rice, maltase is not inherent in this cereal, its presence being due to adherent mould fungi and yeasts (*see* p. 396, vol. ii.). Researches on this point would add to our knowledge on the occurrence of the maltases.

The preparation of "pure" maltase is attended with difficulties. On the one hand, according to E. Fischer (VII.), and Lintner and Kröber (I.), this enzyme is only sparingly soluble in water, and, on the other, it is very susceptible to alcohol, which is generally used as a precipitant for enzymes. Moreover, it cannot be separated from invertase, and therefore, according to Emmerling, the best raw material for maltase is *Schizosaccharomyces octosporus*, which does not contain invertase.

The optimum temperature for this enzyme is given by Lintner and Kröber (I.) as 40° C., whereas that of Geduld's glucase (II.) varies between

57° and 60° C. This difference indicates that the various enzymes decomposing maltose are not identical; and it is therefore advisable to speak of the yeast enzyme as yeast-maltase, and not simply maltase.

The destruction temperature was determined by LINTNER and KRÖBER (I.) as 55° C., which was also confirmed by BAU (XXVI.). Dry maltase has greater power of resisting high temperatures; for, though BOKORNY (IV.) found that the maltase in a pressed yeast was destroyed in the drying process, E. FISCHER (VII.), as well as LINTNER and KRÖBER (I.), had previously ascertained that the enzyme would stand careful drying. According to BAU (XXVI.), the enzyme remains unimpaired when top- or bottom-fermentation yeast is dried at the ordinary temperature, or at 35° 37° C., the dried yeast being then heated for several hours at 105° C. (though in this case the maltase is slightly weakened), or stored for over five years. Bau regards maltase, however, as far more sensitive than invertase to desiccation; so that there is no considerable discrepancy between his statements and those of Bokorny.

The influence of chemical reagents was investigated more particularly by BOKORNY (IV.) in the same manner as for invertase (*see* p. 387, vol. ii.), the results showing maltase in pressed yeast to be more sensitive than that in brewery yeast. The enzyme remained unaffected by the action of 0.5 per cent. solutions of lactic acid and oxalic acid, caustic soda, 0.1 per cent. sulphuric acid and phenol, and by chloroform water (on this point *see* later). It was more or less enfeebled by 0.5 per cent. sulphuric acid, 1 per cent. acetic acid, 0.1 per cent. formaldehyde and thymol, 0.001 per cent. oil of turpentine, and 5 per cent. alcohol; whilst the following agents had a destructive effect: 0.1 per cent. hydrochloric acid, 1 per cent. oxalic acid, caustic soda, or phenol, 0.02 per cent. sublimate, 0.01 per cent. silver nitrate, and 10 per cent. alcohol. By subjecting bottom-fermentation yeast UF to similar treatment BAU (XXVI.) observed a destructive effect on the maltase by 1 per cent. acetic acid, 0.5 and 1 per cent. oxalic acid, 1 per cent. lactic acid, 4 per cent. tartaric acid, 0.5 and 1 per cent. sulphuric acid, 0.91 per cent. hydrochloric acid, 1 per cent. caustic soda, 0.1–0.01 per cent. silver nitrate, and 0.1 per cent. sublimate. The enzyme was also injured by 0.2 per cent. oxalic acid, 1 per cent. sodium carbonate, 0.5 per cent. caustic soda, 0.02 per cent. sublimate, and 95 per cent. (vol.) alcohol. With reference to Bokorny's report (above) that maltase is uninjured by chloroform water, it may be mentioned that, according to MORRIS (III.), fresh, intact yeast, unlike dried yeast, will not decompose maltose. It transpired, however, that MORRIS (IV.) had employed chloroform water to prevent fermentation during the digestion of the yeast with maltose, which reagent, according to EMIL FISCHER (XII.), and also LINTNER and KRÖBER (I.), seriously injures or destroys maltase. EMMERLING (XII.) states that maltase is unaltered by light.

All these experiments show that maltase is a far more sensitive enzyme than invertase. It is apparently unaffected by yeast tryptase, BAU (XXVI.) having found that low-fermentation yeast UF liquefied in five hours at 45° C., exerted a fairly powerful decomposing action on maltose, whilst, on the other hand, no maltase could be detected in expressed yeast juice that had been kept for eight days at about 20° C., or in another sample of the same juice three weeks old. The conditions causing the disappearance of this enzyme were not investigated: and a profitable field is therefore still open for the fermentation physiologist to extend our knowledge on yeast maltase.

Special interest also attaches to maltase, inasmuch as it exhibits not merely hydrolytic properties, but also acts as a synthetic agent. C. HILL (I.) found that, in presence of larger quantities of maltose, the decomposition of this sugar remains incomplete as soon as the solution has become enriched in glucose. Hill prepared his maltase solution by drying low-fermentation beer yeast on earthen-

ware plates and heating the pulverised mass gradually to 100° C., the powder being digested with a tenfold quantity of a weak solution of sodium carbonate for three days, in presence of toluene. The filtrate completely decomposed a 2 per cent. solution of maltose, but not stronger solutions. The presence of glucose also hindered the complete hydrolysis of the maltose. When Hill allowed the maltase solution to act on a 40 per cent. solution of glucose, reversion was observed, 15 per cent. of the sugar being converted into a disaccharide, which Hill regarded as maltose. By means of experiments extending over several months, O. Emmerling (XIII.), however, showed that the reversion sugar is not maltose but isomaltose, namely, Fischer's (XIII.) unfermentable isomaltose, and not that of C. J. Lintner (XLVII.). These two isomaltoses are fundamentally different kinds of sugar, which merely have the same empirical composition, are derived from *d*-glucose and furnish identical phenylosazones melting at 151°–153° C. Unfortunately, we cannot here go into the much-discussed question of the existence of Lintner's isomaltose; but that of Fischer's isomaltose is regarded by Fischer (XIV.) and Ost (II.) as definitely established. Emmerling's claim that Fischer's isomaltose is formed by the action of yeast maltase was disputed by Hill (II.); but, after Emmerling (XII.) had succeeded in reconstructing amygdalin from hydrocyanic acid, oil of bitter almonds, and *d*-glucose by the aid of yeast maltase, Hill (III.) became convinced of the existence of a second sugar, which he named revertose, associated with (Fischer's) isomaltose. On purification, this revertose, or revertobiose, forms strongly hygroscopic, crystalline incrustations, with a specific rotatory power of about $a_D = +91.5°$, and a reducing power equal to only about 47.5 per cent. that of maltose. Revertose needs closer investigation. Perhaps its origin is due to the invertase present in yeast extract. Attention may again be directed here to the circumstance that enzymes, in addition to the possession of hydrolytic or degradation properties, are also able to effect the synthesis of bodies of higher molecular weight, yeast maltase not being alone in the exhibition of this power.

§ 329.—Melibiase.

A polysaccharide known by the names melitose, gossypose, melitriose, and raffinose, with which we shall become more fully acquainted in § 332, can be hydrolysed, by the moderate influence of dilute acids, to two sugars, one of them being the well-known *d*-fructose, whilst the other is a disaccharide with the formula $C_{12}H_{22}O_{11}$, and to which the name melibiose was given by Scheibler and Mittelmeier (II.). Raffinose can also be split up into these two sugars by certain yeasts, Berthelot (X.), for instance, having found that the action of yeast on melitose produces a non-fermentable sugar, which he named eucalyn. According to Bau (XIII.), however, his subsequent reports about this compound are so contradictory that they cannot be utilised in connection with melibiose. In a further communication Berthelot (IV.) states that raffinose (melitriose) is completely fermented by good yeast, but to the extent of only about one-third by enfeebled bakers' yeast. Rischbiet and Tollens (I.) say that melitriose ferments readily and completely; but Loiseau (II.), on the other hand, states that this sugar, whilst completely fermented by low-fermentation beer yeast, is only consumed to the extent of one-third by high-fermentation yeast. Scheibler and Mittelmeier (III.) found that commercial yeast only fermented melitriose imperfectly, and that an amorphous sugar, namely, melibiose, was left in the fermentation residue. In contrast to these experiments, Bau (XIII.) demonstrated that pure cultures of low-fermentation yeasts ferment melitriose completely, whereas those of high-fermentation yeasts effect the separation of a sugar (melibiose) which remains unaltered. This worker (XXVII. and XVI.)

then prepared large quantities of crystallised melibiose, both by the physiological and chemical methods, for particulars of which the reader is referred to the original treatise. As was found by Tollens and his colleague (VI.), and also by Scheibler and Mittelmeier (II.), this disaccharide is split up by the energetic action of acids into two simple sugars, *d*-glucose and *d*-galactose. Hence it contains the same components as lactose (§ 330), but in a different state of chemical combination. According to Bau (XIII. and XV.), mineral acids and oxalic acid alone are suitable for the acid hydrolysis.

Whereas, like lactose, melibiose is only hydrolysed with difficulty by acids, it is readily split up by a yeast enzyme. After Bau (XIII.) expressed the opinion, in 1894, that melibiose is not fermentable direct, but must first be decomposed into its components, *d*-glucose and *d*-galactose, E. Fischer and P. Lindner (II.), as well as Bau (XII.) himself, working independently in the following year, detected in low-fermentation yeast an enzyme capable of effecting this transformation, and to which Bau gave the name melibiase.

Since the occurrence of melibiase in certain *Saccharomycetes* can be utilised as an important chemical means for the differentiation of groups of yeasts, we will now proceed to observe the general characteristics of this enzyme, a knowledge of which facilitates recognition of the value of diagnosing races of yeast on the basis of the action of this enzyme.

Melibiase—which, according to E. O. von Lippmann's (IV.) proposal, should be termed melibio-glucase—is said by Fischer and Lindner (II.), and also by Bau (XII., XXVI., XXVII.), to be somewhat sparingly soluble in water. The optimum temperature at which it decomposes melibiose into one molecule of *d*-glucose and one molecule of *d*-galactose, according to the equation $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$, is 50° C., though a considerable proportion of the disaccharide is split up at much lower temperatures. The destruction temperature is 70° C., though, on the other hand, as ascertained by Fischer and Lindner (II.), melibiase will stand desiccation. According to Bau (XXVI.), low-fermentation yeast that has been dried at 30°–37° C. may be heated to 100° C. for eight hours, or to 110° C. for five hours, without injury to the enzyme; and yeast dried in the above manner will retain its enzymatic activity for $5\frac{3}{4}$ years. In this respect, melibiase has the same power of resistance as invertase.

As regards the action of chemical agents melibiase in yeast is destroyed by the influence of oxalic acid 1 per cent., sulphuric acid 1 and 0.5 per cent., hydrochloric acid 0.91 per cent., sodium hydroxide 1 per cent., silver nitrate 0.1 per cent., mercury chloride 0.1 and 0.02 per cent.; a more or less enfeebling effect being produced by acetic acid 1 per cent., oxalic acid 5 per cent., sulphuric acid 0.2 per cent., sodium carbonate 1 per cent., sodium hydroxide 0.5 per cent., silver nitrate 0.02 per cent., and alcohol 95 per cent. (vol.). Slight injury is caused by oxalic acid 0.2 per cent., and tartaric acid 4 per cent.

With regard to the influence of other enzymes: melibiase is almost as indifferent as invertase. In the method of experiment selected by Bau (XXVI.) —*see* pp. 387 and 390, vol. ii.—the only difference between this and the extremely resistant invertase was that, after the yeast had been treated with pepsin, melibiase could be detected in the filtered-off yeast cells, but not in the filtered solution. Melibiase seems therefore to be rather more sensitive than invertase, though it is far more resistant than maltase. It is also possible that melibiase is insoluble in faintly acid solid solutions, since the filtrate from the pepsin treatment contains free hydrochloric acid; whilst Hill (I.), for instance, found that faintly alkaline water is requisite for the extraction of maltase. Consequently, it must be left for further investigation to determine whether the sparing solubility of melibiase in water can be increased by the careful addition of alkali carbonates.

Melibiase occurs in all low-fermentation types of yeast, both Frohberg and Saaz, an exception being afforded, according to Lindner, by the low-fermentation beer yeasts No. 2, No. 18, and No. 389 of the Berlin collection, these yeasts leaving melibiose "practically unfermented." According to Bau (XXVI.), however, the low-fermentation yeast No. 2 Victoria ferments melibiose, though slowly and sluggishly. Like certain other races, No. 18 is no longer grown in the Berlin collection, and should therefore be struck out of the scientific literature, the rediscovery, and especially the identification, of such yeasts being a matter of very low probability. Yeast No. 389, Grätenthal, does not ferment melibiose, and in this respect forms a remarkable exception among the low-fermentation yeasts, all the others (according to the researches of Bau) containing melibiase.

As a rule the top-fermentation yeasts do not ferment melibiose, Lindner's report that pressed yeasts No. 430, No. 487, and No. 574 decompose this sugar being based on error. On the other hand, melibiose is fermented by the top-fermentation beer yeast, Liegnitz *a* No. 405, and by the pressed yeast, Winterhude, Race III. No. 139. The yeasts, No. 600 and No. 603, from Danish "Jopen" beer ferment melibiose; but contrary to Lindner's report, Broyhan yeast No. 330 (?) does not. These two classes of beer contain numerous organisms that cannot be classed along with culture yeasts; and it is therefore not surprising to find that they contain fungi capable of attacking melibiose. The only true top-fermentation yeasts that split up this sugar are the beer yeast Liegnitz *a* No. 405, and the pressed yeast Winterhude, Race III. No. 139.

In spite of the low-fermentation temperature, low-fermentation yeasts occasionally assume a high-fermentation character, a peculiarity first observed by E. C. Hansen (LXV.)—*see* pp. 194 *et seq.*, vol. ii. The same thing was also noticed by Bau (VI.), at intervals, in Holland; and similar communications have been made by other workers, an exhaustive report having been furnished by W. Henneberg (IV.). The low-fermentation yeast investigated by the latter and found to assume top-fermentation characteristics in a remarkable and constant manner, fermented melitriose completely; and as it also fermented melibiose, it consequently retained the characteristic property of low-fermentation yeast as well. The question therefore arises whether such top yeasts as ferment melibiose were originally low-fermentation yeasts that have acquired top-fermentation characteristics spontaneously and have retained them owing to the conditions of cultivation.

The extensive group of wine yeasts, together with the lactic acid yeasts, do not ferment melibiose. Lindner found that this sugar was attacked by Dürkheim No. 54 yeast and Küster Tokay yeast No. 534. However, since these yeasts are no longer cultivated and no further tests with them are possible, they should be struck out of the scientific literature. With the foregoing exceptions, all the races examined by Schukow (I.), Bau (XXVI. and XXVII.) and Lindner (XXXV.) were found to contain no melibiase, though Kalanthariantz (I.) found wine yeasts that were capable of splitting up melibiose. In one of these races, from Bari in Apulia, a decided hydrolysis of melibiose was observed on digesting the solution of the sugar with the yeast at 40° C., though no such action took place at 25°–30° C. Assmannshausen yeast also hydrolysed melibiose powerfully at 25° C.; but in view of Lindner's (XXXV.) statement that this yeast has no action on melibiose, the report of Kalanthariantz needs confirmation.

Of the wild yeasts that have been accurately defined in a botanical sense, *Sacch. Pastorianus I.* and *III.* ferment melibiose.

A special position is occupied by Logos yeast (*see* p. 203, vol. ii.), which, according to Bau (XV.) and Schukow (I.), does not ferment melibiose, though

Lindner (XXXV.) obtained a different result. According to the results obtained by Bau (XXVI. and XXVII.), there are two races of this yeast, one of them fermenting melibiose, whilst the other does not. Similiar race divisions occur in the case of *Schizos. octosporus*, and *Monilia variabilis*, and especially *Torula colliculosa* (*see* p. 295, vol. ii.).

Summarising these investigations, it appears that, with the exception of Gräfenthal No. 389, all the culture low-fermentation yeasts ferment melibiose, as do also two high-fermentation culture yeasts, namely, Liegnitz *a* No. 405 beer yeast and Winterhude pressed yeast Race III. No. 139, *Sacch. Pastorianus I.* and *III.*, two yeasts from Danzig Jopen beer, No. 600 and No. 603, a number of unnamed wild yeasts and one race of Logos yeast.

According to H. Gillot (V.), melibiose is also left unattacked by top-fermentation yeasts when readily assimilable sugars, such as grape sugar, are presented to the yeast at the same time.

Bau (VII.) based a method for detecting the adulteration of pressed yeast by low-fermentation yeast on the exclusive faculty of the latter for fermenting melibiose.

In the case also of Buchner's permanent yeast, it is possible to detect whether the same is composed of top or bottom yeast, or of a mixture of both, the presence of melibiase being sufficient to demonstrate that of bottom yeast. The test is easily performed by the aid of melibiose, which sugar cannot be split up into its components, *d*-glucose and *d*-galactose, by any yeast enzyme other than melibiase. These components can be readily identified by means of phenylhydrazine, the experiment being carried out in the following manner: A 1 per cent. solution of melibiose, entirely free from any other kind of sugar, is treated with 2 per cent. (or a little more) of the yeast under examination, in presence of 1 per cent. of toluene, and kept for 1–3 days at about 25° C. The extract is filtered, and the filtrate is boiled with a small quantity of good bone black, then refiltered until clear, and the liquid tested with phenylhydrazine, 2 grms. of which, and 2 grms. of 50 per cent. acetic acid, are added for each gramme of melibiose employed, the mixture being heated for an hour on the boiling water-bath. The mixture is poured out into cold water—about 3 vols. to each unit of melibiose solution originally taken—and filtered, the residue being washed once with water and then rinsed into a beaker, in which it is boiled up with water. If the resulting osazone dissolve completely in boiling water, no bottom yeast is present (at least in detectable quantity); but if the osazone remain undissolved, the presence of bottom yeast is demonstrated, since glucosazone and galactosazone are only sparingly soluble in boiling water. In addition to the ratio of solubility in hot water, the microscopical examination affords further indications, inasmuch as melibiosazone crystallises in fine needles, invariably arranged in stellar groups, whereas the two hexosazones chiefly form coarse, long, and thick needles. This difference will be sufficient for the experienced analyst, whilst those who wish to determine the character of the osazone by the melting-point and ultimate analysis must adopt a complicated method—on which point *see* Bau (XV.), who succeeded in detecting with certainty the presence of 10 per cent. of bottom yeast in top-fermentation permanent yeast by this method.

§ 330.—Lactase.

Milk sugar, or lactose, is one of the oldest known sugars, having been described by Fabricio Bartoletti as long ago as 1615. It occurs in the milk of mammals, cows' milk containing 3.6 5 per cent. (mean about 4.5 per cent.), goats' milk 3.26–6.65 per cent., ewes' milk 3.43–6.62 per cent., mares' milk 4.72–7.32 per cent., and the milk of the she-ass 5.29–7.63 per cent.

When lactose is boiled with dilute mineral acids, it is split up into equal molecules of *d*-glucose and *d*-galactose, the hydrolysis, however, being only effected slowly and with difficulty. According to Ost (II.) the reaction is not complete unless one part of milk sugar be boiled with four parts of 2 per cent. sulphuric acid for six hours, or with ten parts of the same acid for four hours. Urech (I.) states that a solution of lactose containing 11.38 per cent. of hydrochloric acid will remain unaltered in the cold, even after the lapse of twenty-eight days; and that it is only when the proportion of acid reaches 32 per cent. that the sugar is almost completely decomposed within twelve hours at 23° C. Milk-sugar solution containing 4 per cent. of oxalic acid will remain unaltered after boiling for eight days; and according to Jones (I.) citric acid also is incapable of hydrolysing lactose. It therefore appears impossible for the lactose in a liquid containing that sugar to be decomposed merely by the organic acids formed by acid bacteria.

Like all di- and poly-saccharides, milk sugar is capable of being fermented directly, but requires to be previously split up into its components by a special enzyme, lactase.

As already mentioned on p. 125, vol. i., Beijerinck discovered this enzyme—to which the name, lacto-glucase, has been applied by E. O. von Lippman (IV.)—in *Sacch. Kefyr* and *Sacch. tyrocola* According to E. Fischer (XV.), lactase cannot be extracted direct from lactose yeast with water, the cells having first to be triturated with ground glass, in order to bring the enzyme into solution. Lactose yeast that has been killed by means of chloroform also exerts a powerful hydrolytic action on lactose solutions. On the other hand, the enzyme can be readily extracted from Kefyr granules by water, on which account E. Fischer proposed to name it kefyr lactase in contradistinction to yeast lactase. This kefyr lactase has greater powers of resistance than maltase to the action of concentrated alcohol.

Buchner and Meisenheimer (I.) recovered from Armenian mazun yeast an expressed juice containing lactase. This yeast lactase will not diffuse through parchment paper, and is therefore—like the enzyme from *Monilia candida* (*see* p. 341, vol. i.)—an endoenzyme.

Among the fungi, *Mucor javanicus*—according to Wehmer (XIII.); *M. Cambodja*—according to Chrzaszcz (I.); and *Allescheria* (*Eurotiopsis*) *Gayoni*—according to Laborde (VIII.)—appear to contain this enzyme. Duclaux (XXX.) found that the matured mycelium of *Aspergillus niger* and *Penicillium glaucum* secretes lactase; but the point needs further investigation (*see* p. 269, vol. ii.).

According to E. Fischer (XV.), emulsin—which in any event is a mixture of enzymes—will also decompose lactose; and Beijerinck (XX.) and Bernheim (II.) report the capacity of a barley enzyme for hydrolysing milk sugar.

Lactase is widely encountered in the animal kingdom, and has been found by Kobert (IV.) in the juices of numerous lower animals. It occurs in the mucous membrane of the stomach and intestines of infants and young animals, according to the statements of Pautz and Vogel (IV.), Weinland (I.), Fischer and Niebel (I.), and other workers.

The expressed juices recovered by Stoklasa and Czerny (II.) from muscle, liver, lungs, and other organs, decompose and ferment milk sugar; and according to Simacek (II.), lactase is also present in pancreatic juice.

According to Weinland, the optimum temperature for lactase is 39° C.; but comprehensive research is needed on this point, as well as on the destruction temperature, and the favourable or adverse influence of chemicals and other substances on the enzyme, these properties of lactase being still undetermined.

Like maltase (*see* pp. 390, 391, vol. ii.), the remarkable property of reversion is also exhibited by lactase, especially that from kefyr. If 200 c.c. of extract kefyr

granules be treated with 100 grms. of *d*-glucose and 100 grms. of *d*-galactose, in presence of 10 c.c. of toluene, and the mixture be kept in a tightly closed flask for fifteen days at 35° C., it is stated by E. Fischer and Armstrong (I.), that the mixture will furnish a new disaccharide, which they term isolactose. This sugar is split up again into *d*-glucose and *d*-galactose by a dilute solution of kefyr lactase, though not by emulsin. A highly interesting observation by E. Fischer and Armstrong is that isolactose is fermented by bottom yeast, but not by top yeast, the sugar thus behaving like melibiose with regard to these two races of yeast.

§ 331.—Trehalase.

The sugar, trehalose, consists of two molecules of *d*-glucose, which, however, are combined in a different manner from the two groups of grape sugar composing maltose, isomaltose, turanose, and probably other disaccharides, such as Hill's (I.) revertose. They are hydrolysed with great difficulty by acids, the decomposition of over 99 per cent. of trehalose necessitating boiling for six hours with 5 per cent. sulphuric acid.

This sugar was formerly regarded as unfermentable, until Böning showed that the same "begins to ferment at the end of twelve hours in presence of best quality yeast that has been sufficiently washed"; compare Tollens (VII.).

Bourquelot (XIII.) afterwards discovered in *Aspergillus niger* (*see* p. 268, vol. ii.), an enzyme decomposing trehalose. For the preparation of this, trehalase—or trehalo-glucase, according to the nomenclature proposed by E. O. von Lippmann (IV.)—he cultivated the mould fungus on Raulin's nutrient solution, triturated the culture with sand, extracted the water by means of 95 per cent. alcohol, and dried the residue *in vacuo*, the mass being then extracted with water and the filtrate precipitated with alcohol. The resulting mould-fungus trehalase, which also occurs in species of *Penicillium* (*see* p. 268, vol. ii.), is destroyed by a temperature of 63° C. It is, however, doubtful whether the yeast enzyme that decomposes trehalose is identical with this mould-trehalase.

Bourquelot also found trehalase in barley and green fodder, and pointed out that the enzyme content, also detected in these raw materials by E. Fischer (XII.), originates in the mould fungi invariably present (*see* p. 389, vol. ii.). The French worker even went further, and expressed the opinion that trehalose is probably only fermented by yeasts when the latter have been grown in unsterilised malt worts, and have thus introduced trehalase derived from the raw material.

According to E. Fischer (XII.), however, pure yeast will hydrolyse trehalose, though invertase and filtered yeast extract do not. The behaviour of various yeast enzymes towards trehalose was then investigated by A. Kalantharjantz (I.), who found that certain wine yeasts hydrolysed 10–21.5 per cent. of trehalose at 22°–28° C., bottom-fermentation beer yeasts attacking 10–37.5 per cent. (at 24° C.), top yeasts, including Weissbier and Lichtenhain yeasts, hydrolysing 5–10 per cent., a number of other species 7.5–25 per cent., Kissly-Schtschi yeast 0 and 20 per cent., Logos 0 on one occasion and 25 per cent. on another, Pombe 0 on two occasions and 5 per cent. once. These results show considerable irregularity in the progress of hydrolysis, especially with the last-named yeasts. According to Delbrück (XIII.), trehalase can be detected in numerous wine, beer, and pressed yeasts.

The researches of Bau (XVIII.) failed to yield any definite result as to the presence, in bottom yeasts, of an enzyme capable of decomposing trehalose. These experiments were conducted at a fermentation temperature of 20°–25° C., and extended over four months. In the case of most of the organisms examined, the fermentation—if occurring at all—started slowly and pursued a sluggish course. The trehalose was gradually fermented by the yeasts US, UF, OS,

Logos, *Sacch. ellipsoideus II.*, and *Sacch. Pastorianus I., II.* and *III.*, as well as by *Monilia candida*, only an inappreciable alteration of the sugar being produced by a lactose yeast, and very little, if any, by *Schizos. Pombe* and *Sacch. apiculatus.*

According to KAYSER (IV.) the pineapple yeast (*see* p. 294, vol. ii.) ferments trehalose, the same effect being produced, according to WENT (V.), by *Monilia sitophila*; by *Allescheria* (*Eurotiopsis*) *Gayoni*, according to LABORDE (VI.); and by the so-called *Amylomyces α* and *Amylomyces γ* (*see* p. 65, vol. ii.), according to ROMMEL and SITNIKOFF (I.).

LINDNER (XXXIV.) states that trehalose is fermented by a yeast from Kissly-Schtschi, by *Monilia candida* and *M. variabilis*, by *Mucor Rouxii*, *Amylomyces*, by Danzig Jopen yeast No. 602, by a race (No. 402) of *Sacch. anomalus*, by nearly all the wine yeasts tried, *Sacch. Pastorianus I., II.* and *III.*, *Sacch. ellipsoideus I.* and *II.*, *Sacch. cratericus*, by two yeasts from Breslau "Kretschmer" beer, and by culture bottom yeasts of the Frohberg type, though no definite result was obtained with Saaz yeast. In the continued experiments with races of the UF type, twenty-four yeasts fermented trehalose, whilst nine others gave negative or indefinite results. A majority of the culture top yeasts fermented this sugar only, rather more than 16 per cent. of them having no effect on trehalose; and similar behaviour was exhibited by most of the wild yeasts, only five out of thirty-seven races giving no result. With regard to Bau's statement that Logos yeast ferments trehalose, Lindner obtained a different result: and whilst Kalanthariantz found that three lactose yeasts split up trehalose, LINDNER (XXXV.) could not obtain any fermentation of this sugar with the same yeasts.

BAU (XVIII.) has pointed out a general resemblance between the fermentation of trehalose and the course of fermentation of saccharose solution by *Monilia candida*, except that the operation proceeds more sluggishly.

Whereas Bourquelot's experiments indubitably prove that certain mould fungi contain an enzyme (trehalase) capable of splitting up trehalose, there does not seem to be any justification for assuming that this is also the case with the true yeasts —so far as present experience extends—the contrary being indicated in a striking degree by the irregularity of the decomposition and extreme sluggishness of the fermentation. The case is probably analogous with the behaviour of *Monilia candida* in presence of saccharose.

§ 332.—Raffinase.

MUDIE (I.) discovered, in eucalyptus manna, a sugar, which was afterwards investigated by BERTHELOT (IX.), who called it melitose. This sugar has been already mentioned on p. 391, vol. ii. When Dubrunfaut, in 1850, observed that certain sugars deposited from beet molasses gave a polarimeter reading of over 100° (in the Soleil-Ventzke-Scheibler apparatus), Scheibler at first attributed this circumstance to the presence of an admixture of dextrin. This hypothesis, however, proved untenable, and the name, "plus sugar," was applied to the constituent causing this higher polarisation in beet sugar. In 1876, Loiseau succeeded in isolating this sugar in a pure state, and named it raffinose, because it was first recovered from sugar-refinery residues. The subsequent exhaustive researches of Tollens and his collaborators, and the simultaneously conducted investigations of Scheibler and Mittelmeier, revealed the identical nature of the sugars, melitose, raffinose, plus sugar and gossypose. The literature of these highly interesting labours—the study of which, in the original, is recommended to all young workers desiring to acquire a knowledge of the characteristics of the various sugars—will be found by referring to BAU (XVII.) and E. O. von LIPPMANN (V.).

Raffinose is a trisaccharide, *i.e.*, it consists of three simple sugars of the C_6 group, namely *d*-fructose, *d*-glucose and *d*-galactose. The two latter are in

a more intimate state of mutual combination—they form melibiose (*see* p. 391, vol. ii.)—than that uniting them with the *d*-fructose. This latter is readily detachable, dilute acids (even weak acetic acid) sufficing to set up hydrolysis resulting in the separation of the trisaccharide into *d*-fructose and melibiose, according to the equation :

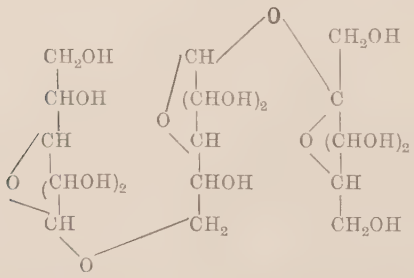
$$C_{18}H_{32}O_{16} + H_2O = C_6H_{12}O_6 + C_{12}H_{22}O_{11}.$$

The quantitative determination of raffinose in presence of saccharose is effected by several methods, which can be found in handbooks on the testing of sugar; and Bau (XIII. and XXVII.) employed a method, based on fermentation physiology, whereby raffinose can also be detected in mixtures with other sugars. By the fermentation method he succeeded in determining the raffinose contained in beet-sugar molasses; but the process requires so much time that it is only suitable for scientific investigations, and not for the practical conditions of the sugar industry.

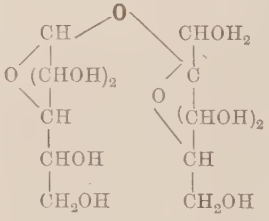
Raffinose is fermented by the great majority of yeasts that are able to ferment saccharose (*see* § 327). The fermentation of this trisaccharide is either complete —as with bottom yeasts—or else only about two-thirds of it are fermented, as is the case with most top-yeasts and a few other kinds, a fission-product, melibiose, being left behind (*see* p. 393, vol. ii.).

It was assumed that raffinose needed to be split up by a special yeast enzyme before it could be fermented; but this opinion was opposed by Bau (XXX.), who asserted that *d*-fructose combines with melibiose to form raffinose, in exactly the same manner as it does with *d*-galactose to form saccharose. The method of combination is shown below, Formula I. representing raffinose, and II. saccharose :

I.



II.



The fructose group on the right in these formulæ is combined in an identical manner with the melibiose group in the one case and with the glucose radical in the other, so that the oxygen combination, indicated by thick type, can easily be resolved by invertase in both instances.

Lindner (XXXV.), however, objected that certain yeasts are able to ferment raffinose, but not saccharose, and that therefore the existence of a raffinase cannot be disputed. As an instance, mention may be made of *Schizosaccharomyces octosporus* which, according to E. Fischer and Lindner (IV.), contains raffinase but not invertase. On the other hand, Lindner's film yeasts No. 170 and 178 will ferment saccharose, leaving raffinose intact. In the case of animal invertase, Pautz and Vogel (I.), as also E. Fischer and Niebel (I.), found that the enzyme from the intestinal mucous membrane of dogs and horses will decompose saccharose, leaving raffinose unaltered.

From these statements, the existence of raffinase may be assumed to be demonstrated; but all that we know about it, apart from the fact that it splits up raffinose into *d*-fructose and melibiose, is that—as observed by Bau—it is

decomposed at a temperature of 75° C., and is contained in Merck's invertase. According to Gillot (V.), raffinase occurs in mould fungi, as well as in yeasts.

In the case of polysaccharides, according to Bourquelot (XIV.), the enzymes act successively, so that in our case, the raffinose must first be hydrolysed by raffinase, before the melibiase can act on the liberated melibiose. No reversion takes place, and melibiase cannot attack raffinose itself.

In addition to raffinase—which merits exhaustive investigation—there are certainly other enzymes that are able to split up the polysaccharides; but as even less is known about these enzymes than about raffinase, we cannot devote a separate paragraph to them, and therefore they are briefly reviewed below.

Thus, it is doubtful whether the manna-trisaccharide, which Tanret (VI.) found in the manna of the ash-tree, is partially or completely hydrolysed by yeast, or not attacked at all.

The reports on the fermentation of melecitose by yeasts also require confirmation. According to Bourquelot and Hérissey (III. and V.), this sugar is decomposed by an enzyme of *Aspergillus niger*, into glucose and turanose. This latter sugar, a biose, composed of two *d*-glucose groups, may also be formed by the hydrolysis of melecitose by acids.

Bourquelot (XV.) states that only one-third of gentianose is fermented by yeast, the latter separating *d*-fructose and fermenting that sugar, but leaving the gentiobiose behind. However, under the influence of an enzyme of *Aspergillus niger*, and also of emulsin, gentiobiose can be split up, by further hydrolytic action, into two molecules of *d*-glucose.

A tetrasaccharide, stachyose, which was specially investigated by Tanret (VI.) and by A. von Planta and E. Schulze (I.), is also only partially fermented by yeast, *d*-fructose being liberated and fermented, leaving a trisaccharide behind. Whether the latter—known as mannotriose—can be in turn attacked by yeast, has not yet been accurately determined. Emulsin, diastase, and *Aspergillus niger* act on stachyose in the same manner as yeast enzyme. Our knowledge of intimate properties of these enzymes that decompose the last-named polysaccharides is practically nil; and their investigation would therefore be extremely valuable, though complicated by the difficulty in the way of procuring the higher polysaccharides.

§ 333.—Fermentation of Dextrin by Yeast (Amylase).

As long ago as 1833, Biot and Persoz (I.) expressed the opinion that dextrin can be fermented by yeasts; though one of them, Persoz (I.), in collaboration with Payen, came to the conclusion, in the following year, that dextrin was unfermentable. This view was shared by G. Varry (I.), whereas Barfoed (I.) arrived at the opposite result.

In the course of their researches on the degradation of starch by diastase, Brown and Morris (IV.) found that dextrins are not directly fermentable by yeasts, but require first to be hydrolysed. Under certain conditions a few bottom yeasts, which act as the cause of secondary fermentation in English top-fermentation breweries, and are referred to by the authors in question as *Sacch. ellipticus* and *Sacch. Pastorianus*, are capable of hydrolysing dextrin, and thus afford an impetus to the direct fermentation of that substance. According to E. R. Moritz (I.), maltodextrins are not fermented during primary fermentation, but constitute the material for secondary fermentation.

According to the conception of the English chemists, the starch molecule is degraded, under the influence of diastase, in such a manner that a series of dextrins, the so-called amyloins, are formed, these constituting intermediate stages between soluble starch and maltose. These amyloins, which are also

occasionally known as maltodextrins among German workers, are constituted, according to the theory of Brown and Morris (V.)—in such a manner that m molecules of maltose, $C_{12}H_{22}O_{11}$, are combined with n molecules of dextrin, $C_{12}H_{20}O_{10}$. In the amyloins of low type, resulting from the more extensive degradation of the starch, the value m is far greater than the value n, the converse being the case with the amyloins that approximate more closely to starch in their composition. This clever hypothesis, however, failed to stand further theoretical and practical investigation; in which connection we need only mention the work of C. J. Lintner, who arrived at a different result in numerous published researches. In one of their experiments, Lintner and Düll (II.) found that the primary degradation-product of starch is an amylodextrin, which is degraded into erythrodextrin by the action of diastase. Under the hydrolytic influence of malt enzyme, this product is transformed into achroodextrin, which furnishes maltose and Lintner's isomaltose—not to be confounded with Fischer's isomaltose, which is a well-defined, synthetic, non-fermentable sugar (*see* p. 391, vol. ii.). Varying results were obtained by other workers, both earlier and later; in fact all those who have taken up the problem of the degradation of starch by diastase—a question that has been under discussion for more than a century—have obtained different results, so that in the mouth of one author, the relatively established terms, amylodextrin and erythrodextrin, and more especially achroodextrin I., II., III., maltodextrin, &c., meant different substances to those implied by a second or third author, who believed himself to be working with the same compounds. An almost irremediable confusion exists in the nomenclature of these products, owing, no doubt, to the inadequate chemical and physical appliances at our disposal to enable the several transformation products to be isolated as well-defined individuals. Perhaps the exhaustive work of Moreau (I.) may finally clear the matter up. It would take up too much space to give even a brief historical review of the various hypotheses dealing with the intermediate products between starch and maltose; and we must therefore be content in this paragraph to class as "dextrin" all the intermediate bodies between starch and maltose that different authors have named amylo-, erythro- and achroodextrins, maltodextrins and amyloins, moreover, without drawing any distinction between the dextrins produced by diastase and those resulting from the action of acids on starch.

According to the views of English chemists, the amyloins effect the secondary fermentation of beer in the storage cask; which view is also expressed by E. R. Moritz (I.). If low-type amyloins be added to beer, the secondary fermentation assumes a more turbulent aspect and the beer matures earlier. In the opinion of Moritz, secondary fermentation is not effected by the true *Saccharomycetes*, but by the secondary-fermentation yeasts, with which Brown and Morris associate wild yeasts. This view was responsible for the sceptical attitude taken up in England with regard to the application of the Hansen method of pure-yeast culture to the production of top-fermentation beers. According to Moritz, the higher-grade amyloins, which dextrins approximate more closely in composition to starch, cause gradual secondary fermentation, so that the beer takes longer to mature.

Medicus and Immerheiser (I.) were led into quite a different sphere of labour on the fermentability of dextrins, by the examination of a suspected wine for the presence of added impure starch sugar. In their experiments on the fermentation of dextrin by yeasts they employed, in part, impure starch sugar, and in part prepared from this latter a so-called dextrin, which they subjected to the action of commercial, but starch-free, pressed yeast. For example, 40 grms. of starch sugar were dissolved in 250 c.c. of water containing

nutrient salts, the glucose being eliminated by fermentation. The resulting solution, corrected to the original volume, gave a polarimeter reading of $+7.65^{\circ}$ in the first experiment, and $+6.90^{\circ}$ in the second. After pitching the filtered solution with 5 grms. of pressed yeast, the polarimeter readings at the end of 6 days receded to 3.35° and 2.75° respectively; and after a further sojourn of 5 days at 30° C. the reading had fallen to 1.9 in both cases, an additional diminution ensuing after the addition of more yeast. In other experiments the same workers succeeded in eliminating the dextro-rotatory substances completely; but the process cannot be regarded as a true fermentation of dextrin in our sense of the term, neither carbon dioxide nor alcohol being produced.

E. von Raumer (I.) also, in the course of examining certain honeys suspected of containing added starch sugar, endeavoured to ascertain how far dextrins can be fermented by yeasts. His results showed that pressed yeast, beer yeast and wine yeast behave differently, the first named attacking the dextrins powerfully, the latter not at all.

Fresenius (III.) was able to confirm the reports of Medicus and Immerheiser. According to his researches the so-called unfermentable dextro-rotatory substances are also fermentable by pressed yeast, though not affected by beer yeast. Fresenius, moreover, drew attention to the circumstance that film yeasts also eliminate dextrins.

Hence, it is important for the analyst who has to examine honey, or wine for added starch sugar, to know that he should never employ commercial pressed yeast. The "Convention," Vereinbarungen (II.), prescribes that 25 grms. of honey be dissolved in 200 c.c. of a nutrient solution and sterilised, and after cooling should be pitched with 5 c.c. of fluid, vigorous and, preferably, pure-culture wine yeast, *not pressed yeast*, fermentation being conducted at 20°–25° C. The liquid is then made up to 250 c.c. with water, after being clarified with alumina, or 3 c.c. of lead acetate and 2 c.c. of sodium sulphate solution, and is examined in the polarimeter at 20° C. If the fermentation residue exhibits any considerable dextro-rotation, it should be examined for dextrin by precipitation with alcohol.

In the examination of wine for the presence of impure starch sugar, K. Windisch (VI.) recommends that 210 c.c. of the wine be concentrated to one-third by evaporation, and then diluted with water until the liquid contains not more than 15 per cent. of sugar. This solution is pitched with about 5 grms. of vigorous beer yeast that is free from optically active constituents, and fermentation is carried out to completion at a temperature of 20°–25° C. The fermentation residue is tested for dextrin by a special method given by the same author (*l.c.*).

Lintner (XII.) opposed the views of Medicus, Fresenius and others by asserting that the earlier experiments were inconclusive, having been performed with impure dextrin or impure yeast. The dextrins in starch sugar are partly reversion products, such as the gallisin of Scheibler and Mittelmeier (IV.); and the pressed yeasts are not composed of uniform organisms, but contain many bacteria capable of attacking dextrin. Malt dextrin is unfermentable by pure yeast of *Sacch. cerevisiæ* type; and the numerous conflicting reports may also be explained by the circumstance that many of the workers employed dextrins containing sugars, and not the pure substance. E. von Raumer's observation of a difference in yeasts, inasmuch as pressed yeast attacks dextrin most powerfully, beer yeast acting in moderate degree, and wine yeast not at all, renders it fairly certain that his pressed yeast contained large numbers of bacteria, and the beer yeasts probably contained film yeasts, whilst the wine yeast was relatively pure. Shifferer (I.) also reports that malt dextrins resist

the action of pure top yeast, though they are eliminated by treatment with impure commercial pressed yeast.

A new stage in our knowledge of dextrin fermentation was reached when the study of yeasts led to the establishment of certain definite types in the *Sacch. cerevisiæ* group. This was stimulated by the discovery of Saaz yeast (*see* p. 81, vol. ii.) and the differentiation between the Saaz and Frohberg types, the former of which gives a lower final attenuation than the latter. Since both top and bottom yeasts form well-defined races, we have the types: bottom Frohberg, bottom Saaz, top Frohberg, and top Saaz, distinguished by the signs: UF, US, OF, OS respectively. Yeasts of the OF and UF type produce the same final attenuation in beer wort, OS and US being also equal in this respect. It is true that insignificant fluctuations occur in the final attenuation, since the yeasts do not merely ferment the carbohydrates, but also assimilate and modify a number of other wort constituents, especially nitrogen compounds and salts, besides producing non-volatile products, especially glycerine and succinic acid (*see* p. 315) in varying quantities. This explains why the residual extract in one and the same wort that has been fermented by different yeasts, *e.g.*, of the UF type, is not identical in all cases, but frequently exhibits small differences.

In addition to the foregoing, H. van Laer (III.) established the Burton type of yeast, which ferments worts to a greater extent than the UF yeasts. He also differentiated intermediate types, giving an attenuation midway between the types already mentioned, and named them Saaz-Frohberg and Frohberg-Burton. These types, however, never found acceptance in fermentation physiology; and, in fact, van Laer (XII.) himself partly abandoned them on discovering Logos yeast. He then distinguished between the types S, F, and Logos, as well as the subsequently discredited intermediate types, Saaz-Frohberg and Frohberg-Logos.

A new type was discovered by Lindner (XXX.) in *Schizos. Pombe* (*see* p. 216, vol. ii.), which is able to carry the fermentation of wort further than is the case with Logos yeast. This yeast has been exhaustively studied by W. Windisch (VI.) and F. Rothenbach (I.).

Consequently we have the following four types of yeast: S, F, Logos, and Pombe. The differences of attenuation produced by these yeasts are undoubtedly due to the fact that some of them are able to ferment dextrins, in addition to maltose (and hexoses). This faculty has been proved in the case of Logos yeast and *Schizos. Pombe*, though the cause of the difference between the types S and F has not yet been established on a scientific basis. It is true that numerous workers, especially Prior (VII.), have occupied themselves with this problem; but, up to the present, the researches and hypotheses of Prior, and also those of Bau (XXXII.), have not led to any final and recognised result.

The direct examination of the fermentability of dextrin by pure cultures of yeast was performed by Lindner (XXXV.), who obtained the surprising result that many species ferment dextrin in a greater or smaller degree, even yeasts of the Saaz type being found by the same author (XLVII.) to be capable of attacking dextrin, namely, the top-fermentation OS yeasts, Nos. 150, 159, 160 and 403. On the other hand, Saaz yeast itself, and various other races belonging to the OS and US types, do not ferment dextrin. Certain other top-fermentation beer yeasts of the OF type, including Burton yeast, attack dextrin; and indeed, out of forty yeasts of this type examined, only eight left the dextrin intact.

Nearly all top yeasts from pressed-yeast factories ferment dextrin energetically, only one yeast from Bavaria, out of thirteen races examined, giving a doubtful result—compare Lindner (XLVII.).

The true distillery yeasts, on the other hand, have, in general, little or no fermentative power on dextrin, 50 per cent. of the races examined leaving this carbohydrate intact.

Out of thirty-three low-fermentation beer yeasts, only one fermented dextrin energetically, another slightly; and in the case of fourteen races the result was doubtful—compare LINDNER (XLVII.), who also reports that a few races of wine yeast can ferment dextrin.

The film yeasts examined by Lindner have no action on dextrin; but, on the other hand, the species of the genus *Willia* (*see* p. 213, vol. ii.) seem to attack this carbohydrate slightly, only one species, *W. belgica*, giving decidedly negative results.

The wild yeasts with botanical names, *S. Pastorianus I., II., III.*, *S. ellipsoideus* and *S. cratericus*, ferment dextrin more or less strongly; of thirty-one other wild races examined, thirteen pure cultures were found to liberate carbon dioxide, faintly or decidedly, from dextrin solutions.

Of other organisms that ferment dextrin, mention may be made of: *S. exiguus*, *Monilia candida*, *Mon. variabilis*, *Sachsia suaveolens*, *Mucor Rouxii*, *Amylomyces* β and *Am.* γ, Logos yeast, *Schizos. Pombe*, *Schizos. mellacei*, and *Schizos. octosporus*. This carbohydrate is also attacked by a series of yeasts from Danzig Jopen beer.

According to P. Lindner's researches, no definite rule exists with reference to the fermentability of the dextrins by special yeasts; and it is urgently necessary that these investigations should be continued under the conditions laid down by C. J. Lintner, namely, the use of pure cultures and pure dextrins exclusively. The former condition was fulfilled by Lindner, but the solution of the second problem is complicated by the circumstance that, in spite of all that has been done, the dextrins resulting from the action of diastase on starch have not yet been isolated in the form of scientifically pure chemical entities.

However this may be, we may assume that the dextrins are not directly fermentable by yeasts, but that, like all polysaccharides, they must first be split up into directly fermentable sugars by the action of an enzyme. This enzyme, which has been named amylase, was found by KATZ (II.) in various mould fungi, including species of *Penicillium* and *Aspergillus* (*see* p. 261, vol. ii.), as also in *Bact. megaterium*. Our knowledge of the amylase of yeast is at present very scanty, and much work is needed to amplify it.

Having now become acquainted with the present state of affairs with regard to the fermentation of dextrin, we are able to understand the patent taken out by EFFRONT (XV.) for the cultivation of yeast possessing the power of fermenting that substance. According to the inventor, certain beer yeasts that are capable of slightly fermenting dextrin can have their powers in this respect considerably augmented by cultivation under favourable conditions. The yeasts are first grown in a medium that contains aldehyde, in addition to dextrose and mineral substances (potassium nitrate). After preparation in this manner, they exhibit a decided tendency to ferment dextrin; and this property can be further intensified by suitable treatment. The scientific explanation of the Effront process may be sought in two directions; either each beer yeast contains a dormant enzymogen, which can be stimulated to an increased production of amylase by suitable treatment, or else the method of cultivation (perhaps by spontaneous infection) favours certain yeasts that already produce amylase, so that they gain predominance in the cultures, under the conditions of environment selected by Effront, obeying the law of natural pure culture as established by Delbrück. No accurate investigation has yet been instituted on either of these lines; and up to the present nothing is known as to the practical success of the Effront method.

§ 334.—The Autofermentation of Yeast.*

Except for an antecedent, though indefinite, announcement by Berthelot (III.), Pasteur may be regarded as having been the first to observe that, under certain conditions, the total carbon dioxide and alcohol produced from a solution of sugar by the action of yeast may exceed the theoretical yield calculated from the sugar initially present in the nutrient solution. Thus, for instance, 0.424 grm. of sugar and 10 grms. of yeast (calculated to dry substance), instead of furnishing about 110 c.c. of carbon dioxide, in accordance with the equation of decomposition, actually produced 300 c.c., together with 0.6 grm. of alcohol. Since only a portion and not the whole of this yield is covered by the quantity of sugar present in the nutrient solution, the remainder must have originated in the constituents of the yeast cells, so that the latter possess the faculty of fermenting, not merely the sugar in their vicinity, but also the material of their own corpus. As a matter of fact, under certain conditions (previous abundant nutrition, restriction of the access of air, &c.), considerable quantities of alcohol may be formed in a mass of yeast, without even a single trace of sugar having been added. The term applied to this phenomenon is "autofermentation."

Pasteur's explanation of the phenomenon was that the yeast cells contain a substance capable of being transformed into sugar and afterwards fermented; and, by boiling yeast with dilute sulphuric acid, he succeeded in extracting no less than 20 per cent. of fermentable sugar (calculated on the dry matter of the yeast). His assumption, however, that the cell wall is the source of this sugar was an error out of which his rival, Liebig (III.), made a good deal of capital. This latter worker showed, in the case of a number of samples of yeast, that the cellulose, forming the residue of the method of extraction employed at that time, was in considerably smaller amount than would be requisite to produce the total alcohol resulting from autofermentation. Thus, to mention only a single instance advanced by Liebig, 100.6 grms. of yeast, found by a preliminary experiment to contain 13.9 per cent. of so-called cellulose, furnished by autofermentation 13.9 per cent. of alcohol, whereas the theoretical yield from the amount of cellulose in question was only 11.3 per cent. at the most. Liebig regarded this observation as a proof of his own theory of fermentation; but it must be pointed out that, though the determination corrected an erroneous (but by no means fundamental) error of his French opponent, it did not controvert the latter's explanation of the nature of autofermentation. The counter-hypothesis successfully brought forward against Liebig by Nägeli and Loew (II.), namely, that yeast contains a greater amount (up to 37 per cent.) of cellulose than reported by him is, however, untenable for the reason that it was based on the result of analytical determinations in which the so-called cellulose was not weighed separately, but was taken in conjunction with a mixture of other mucilaginous substances (*see* p. 127, vol. ii.), probably including glycogen, which at that time had not been identified.

After Salkowski (IV.) had observed that yeast digested with chloroform water does not undergo autofermentation, but yields a lævo-rotatory sugar, he arrived at the conviction that the source of this sugar was to be found in glycogen. The same result was attained by Cremer (I., V. and VII.), except that this worker showed the resulting sugar to consist of the dextro-rotatory *d*-glucose. The accuracy of this latter point was subsequently admitted by Salkowski (IV.).

* This paragraph has been chiefly drafted on the reports of Professor Lafar, to whom I am greatly indebted for his valuable assistance here, and in other portions of chapters lxiv. and lxv.—A. B.

Autofermentation, therefore, proceeds at the expense of yeast glycogen, the degradation of this carbohydrate being accompanied by other transformations that were formerly regarded—and also by KUTSCHER (II.)—as autofermentation. These phenomena relate to the transformation of the nitrogen compounds. According to M. SCHENCK (I.), however, a sharp distinction must be drawn between the autofermentation and the autodigestion of yeast. This latter form of decomposition will be dealt with in chapter lxvi.

Nevertheless, it will be advisable to mention here that aromatic perfumes are formed during autofermentation, probably as a direct accompaniment thereof. These fruity odours are emitted with special intensity when the yeast is stored in a pressed state, a fact well known in practice. Further investigations on the nature and causation of these aromatic substances are desirable.

Some particulars have already been given (p. 123 *et seq.*, vol. ii.) respecting glycogen and its hydrolysis by a yeast enzyme. This latter, to which the name glycogenase, has been given, seems to be incapable of diffusion through the cell membrane of the yeast, its action being confined to the interior of the cell. This may be concluded from the circumstance that the liberated enzyme in expressed yeast juice prepares added glycogen for fermentation. This glycogenase, which to some extent is still of a hypothetical character, is credited not merely with the faculty of hydrolysis but also, in a high degree, with the power of reconstructing glycogen under certain conditions. With regard to maltase and lactase it has already been mentioned, on pp. 390 and 395, vol. ii., that these two enzymes are capable of producing reversion effects in addition to their hydrolytic properties. The condition under which glycogen increases or disappears in the yeast cell have been exhaustively investigated by HENNEBERG (II.); according to his view (VI.) it may be assumed that *Sacch. apiculatus* does not contain glycogen. Further investigation on the subject of glycogenase would constitute a valuable sphere of labour, since all that can be predicted with regard to its still hypothetical existence is the possession of the two properties mentioned.

A few remarks may be made here respecting the manner in which autofermentation may be influenced by various agencies. We have already seen on pp. 125, 126, vol. ii., that, according to Salkowski and Cremer, yeast is affected, by digestion with chloroform water, in such a manner that, whilst the glycogen is split up, the yeast does not undergo autofermentation. C. J. LINTNER (III.) states that the same effect can be produced by the addition of common salt, and he also investigated the action of other saline substances by adding, in all cases, 5 grms. of the salt under examination to each 10 grms. of aspirated bottom yeast rich in glycogen, containing about 25 per cent. of dry substance. Autofermentation was not set up in the samples treated with a chloride of sodium, magnesium, or aluminium, or with ammonium chloride, nitrate, or sulphate. A restrictive influence was produced by the sulphates of manganese and copper, as well as by potassium nitrate. On the other hand, a stimulating effect was brought about by the sulphates of sodium, zinc, magnesium or ferrous oxide, and by monopotassium phosphate. Hence, in certain circumstances, autofermentation may fail to occur in a yeast that is very rich in glycogen, when treated with restraining agents.

The autofermentation of yeast had a certain practical value for the analytic chemist, namely, when the sugar content of a sample submitted for examination is to be determined by the physiological method (*see* p. 321, vol. ii.). It is true that methods are available in which the influence of the yeast can be minimised, not only with regard to autofermentation, but also with reference to the other transformations (*see* pp. 359 and 380, vol. ii.) brought about by yeast, the sterilised solution being inoculated with an imponderable trace of yeast, as recommended by ELION (V.) and BAU (XXXI.). These methods, however, occupy a good deal of

time, and though the time question may be neglected in researches of a purely scientific character, it prevents their application in practice, despite their accuracy. For a rapid examination, or commercial analysis, a larger sowing must be used, and in these circumstances the glycogen content and autofermentation of the yeast must be borne in mind, no matter whether the sugar be determined from the resulting carbon dioxide or the alcohol formed. These derivatives are neglected in the method of Elion and Bau, the only determination made being the loss of extract during fermentation, the result, supplemented by the cupric reduction and polarimeter tests, giving the actual content of sugar. In commercial analyses a blank experiment has been recommended, in which the same quantity of yeast is employed with the liquid under examination in the one case, and with an equal volume of distillled water or nutrient solution free from sugar in the other. If the sugar content be determined from the carbon dioxide liberated, it has been thought that these precautions would eliminate the influence of the autofermentation of the yeast. The experiments of C. J. Lintner, however, have shown that the saline matters present act differently on autofermentation; and therefore, as the full composition of the liquid under examination is generally unknown, it is impracticable to carry out the blank experiment in such a manner that the yeast acts in a liquid that is identical with the one in question, except that sugar is absent. In the blank experiment one is working with unknown quantities, whose influence on autofermentation is still undetermined, so that new sources of error are introduced to an indefinite extent. It has been proposed to stop the fermentation experiment at the point at which a preliminary test has shown that the sugar of the counter experiment is completely fermented; but, as was seen on p. 126, vol. ii., the degradation of the glycogen begins at a time when fermentable sugars are still present in the nutrient medium. According to Jodlbauer, this danger does not exist when not more than two parts of moist yeast (containing about 25 per cent. of dry substance) are used to one part of sugar. The method recommended by him for the physiological determination of sugar is as follows: The nature of the sugar present in the samples is first ascertained by qualitative tests; and then the reducing power of the substance toward Fehling solution is determined (after hydrolysis in the case of saccharose or raffinose; this method being inapplicable in the case of trehalose), and from the results so obtained a calculation is made of the amount of substance that contains 2 grms. of the sugar. This amount—which in the case of solid substances is dissolved in 25 c.c of water, is treated with 1 grm. of fresh beer yeast, that has been freed from water on an unglazed porcelain or earthenware plate, 1 c.c. of Hayduck's nutrient solution being added when the substance is low in nutritive substances. Fermentation is allowed to proceed at about 34° C., a weak current of hydrogen gas being drawn through the liquid, and the escaping carbon dioxide collected in an absorption apparatus. If the continued examination of a parallel experiment justifies the assumption that all the sugar in the main flask has just been consumed, then the main experiment is stopped, and the whole of the carbon dioxide remaining in the liquid and the free space of the flask is driven over into the absorption apparatus by careful boiling and the continued passage of a small current of hydrogen, the amount of the fermented sugar being determined from the increased weight of the absorption apparatus. In spite of all the excellent work performed by Jodlbauer in this connection, it must be admitted that this method should only be resorted to when all other means have failed. The apparatus must be put together with great care in order to prevent, on the one hand, any escape of fermentation carbon dioxide throughout the whole of the experiment—lasting about twenty hours in presence of glucose, and twice as long in the case of saccharose—and, on the other, to exclude atmospheric carbon dioxide.

These particulars should also be borne in mind by those who wish to determine

the amount of sugar in urine by the fermentation method. More precise information on the method employed for this purpose and the Einhorn saccharometer—which is similar to the fermentation flask noticed on p. 159 of vol. i.—may be found in the handbooks on Urine Analysis, especially that of Neubauer and Vogel, the last three editions of which have been supervised by H. Huppert (I.). All these handbooks advise a check experiment, to ascertain the amount of carbon dioxide liberated by the yeast on digestion with water only. Though the question of auto-fermentation is thus touched upon, the prescriptions given leave much to be desired on the score of accuracy, and in particular fail to bear in mind the possible occurrence of glycogen in the yeast. Excellent service in this connection has been rendered by E. Büchner and S. Mitscherlich (I.) by the elaboration of a method of preparing yeast free from glycogen. Utilising the observations of Henneberg (IV.), they treated yeast by spreading the pressed and screened material in a thin layer exposed to the air. On the yeast being kept in the ice-chest (at about 2° C.), no glycogen can be found after about a day; at about 20° C. it disappears in eight hours; and as quickly as 3–4 hours in the thermostat at 35°–45° C. As a rule the fermentative power of the yeast is unimpaired by this treatment. These workers state positively that yeast as free as possible from glycogen must be used for the detection of sugar in urine, since yeasts that are rich in glycogen may give rise to the erroneous impression that the urine under examination contains sugar. The permanent yeast sold under the name "zymin" is not suitable for this purpose, inasmuch as it contains glycogen (*see* p. 353, vol. ii.).

In conclusion it may be mentioned that the value of many of the scientific treatises dealing with the attenuation powers of yeast will assume a different proportion when examined with a view to ascertaining whether the possibility of the intervention of autofermentation has been duly considered by the authors. All experiments in which increased quantities of yeast have been employed in one and the same test require to be repeated and confirmed in this connection.

CHAPTER LXVI.

ENDOTRYPTASE AND PHILOTHION.

By Dr. M. HAHN (§ 335) and Dr. LAFAR (§ 336).

§ 335.—Endotryptase.

The existence of a proteolytic enzyme in yeast was indicated by the discoveries of several of the earliest workers, attention to the subject being drawn more particularly to the phenomena occurring in autofermentation (p. 404, vol. ii.). After it had been shown by Thénard (I.), Pasteur (XXXIV.), and Duclaux (XXXI.) that yeast loses weight during fermentation, and especially becomes poorer in nitrogen, Liebig (II.) discovered the presence of leucin in water surrounding yeast that had been undergoing autofermentation. Béchamp (VII., VIII., X., XI.) and Schützenberger (II.) were the first who learned to differentiate two separate processes in autofermentation: one leading to the decomposition of the carbohydrates into alcohol and carbon dioxide, and the other resulting in the decomposition of proteids and therefore worthy to rank as an actual digestion process. Béchamp's discovery of the hydrolysed proteids in the water used for washing yeast led him to formulate a physiological theory of fermentation: "In yeast, as in the case of all living organisms, we observe a double series of phenomena. First the phenomena of nutrition and assimilation induced by the presence of their foodstuffs (sugar, nitrogenous substances, and mineral salts), these various substances entering the cells endosmotically and being there transformed and utilised in the construction of tissues for the newborn cells. Side by side with these phenomena of nutrition, however, but reversed, occur the phenomena of disassimilation, whereby the tissues are transformed into excrementitious substances, which are no longer beneficial to the life of the cell, and are ejected." (He classes alcohol and carbon dioxide in this category.) More recently, Boullanger (I.), Beijerinck (XXXI.), Artari (I.), Wehmer (XII.), and especially Will (XXXV.) have more closely investigated the proteolytic processes in yeast cultures, after the theory of the autodigestion (autolysis) of yeast has been firmly established by Salkowski (II.), by the digestion of yeast in chloroform water (*see* pp. 125, 126, vol. ii.). M. Hahn (I.) succeeded in preparing a cell-free solution of the enzyme, after demonstrating the presence of a strongly proteolytic enzyme in the expressed juice obtained from yeast by the method of Buchner and Hahn (*see* p. 342, vol. ii.), and studying the properties of this enzyme (which he named "yeast endotryptase") in collaboration with L. Geret (I. and II.). The fission products of autodigestion were afterwards more closely examined by Fr. Kutscher (III.), chiefly by the Kossel method.

The existence of endotryptase can be proved in a most convenient and striking manner with yeast juice prepared by the method of Buchner and Hahn. A few cubic centimetres of the expressed juice distributed on thymol- or carbol-gelatin (*see* p. 231, vol. i.), or an ordinary nutrient gelatin with an addition of thymol, in a test-glass, produce a decided liquefaction of the gelatin in

twenty-four hours at 22° C., the whole being liquefied in two to three days when 10 c.c. have been taken. The autolysis of the pressed yeast juice is equally convincing and undeniable; whereas the freshly prepared juice is strongly coagulated by boiling, a decided diminution of the coagulum is observed on boiling the juice after it has been kept, in presence of a little toluol or chloroform, for twenty-four hours at 37° C., a precipitate, however, forming without boiling. The formation of a coagulum is, moreover, almost entirely prevented by storing the juice for six to seven days at 37° C., or ten to fourteen days at room temperature; whilst a deposit of amino-acids (leucin particularly) is formed. Of course the dried pressed juice, mentioned on p. 345, vol. ii., can be used for the same purpose, after being dissolved in water and treated with an antiseptic. The detection of endotryptase can also be effected with the recently introduced permanent yeast (*see* p. 358, vol. ii.) on stirring the latter up to a thin pap with water and spreading it on gelatin in presence of toluol. This method, however, never produces such rapid liquefaction as can be obtained by the use of expressed yeast juice. Carmine fibrin (*see* p. 231, vol. i.) suspended in pressed yeast juice also dissolves in twenty-four hours at 37° C., and stains the liquid dark red; but the liquefaction of coagulated egg albumen takes longer to accomplish. Will (XXXI.) also observed the liquefaction of gelatin in living cultures, with stab cultures of various species of *Saccharomycetes* (*see* p. 413, vol. ii.) in wort-gelatin, and kept for eighteen to eighty days at 20° C., or for forty-five to two hundred and forty days at 13° C., the liquefaction usually beginning in the path of the stab. The quantitative determination of the effect produced by endotryptase can also be performed in a most convenient manner with pressed yeast juice, the coagulum produced by boiling the fresh and digested juice being dried and weighed, or else (which is preferable) the increase of nitrogen in the filtrate is ascertained. With this object 10 c.c. of yeast juice are diluted with water, treated with about 5 c.c. of a saturated solution of common salt, neutralised, heated to boiling, acidified with a few drops of acetic acid, and filtered through a dry filter after having been made up to a definite volume on cooling. The nitrogen in an aliquot part of the filtrate is then determined by the Kjeldahl method. By performing the determination with fresh and digested juice, the increased amount of nitrogen in the filtrate gives a very accurate representation of the progress of digestion. According to Salkowski (II.) the determination is effected in precisely the same manner in the case of yeast suspended in water, the nitrogen being determined in the filtrate (separated from the coagulum) before and after the digestion of the yeast in water containing a little chloroform or toluol. The proteid nitrogen may be determined either in the coagulum, or else, according to Iwanoff (II.), in the precipitate furnished by copper oxide (*Stutzer*).

Chief among the properties of endotryptase ranks its practically valuable behaviour toward high and low temperatures. Temperature is the decisive factor, not only for the decomposition of proteids during fermentation (as already mentioned several times in chap. lxiii.), but also for the activity of alcoholase, which enzyme may, in certain circumstances, be injuriously affected by the endotryptase that is acting concurrently. All experiments on the properties of endotryptase may be carried out in a most satisfactory manner with expressed meat juice which, to some extent, represents a solution of this enzyme, and forms the best material from which the action of the enzyme can be quantitatively determined. Geret and Hahn obtained the accompanying particulars (*see* Tables on next page) with regard to the optimum and destruction temperatures of the enzyme.

The optimum temperature seems therefore to lie between 40° and 45° C., whilst the enzyme is completely destroyed by heating at 60° C. for an hour. In

Temperature ° C.	Percentage of Coagulum.	
	Before digestion.	After digestion for 20 hours.
1. At 3°–7°	2.72	2.57
2. „ 22	4.71	3.93
3. „ 37	4.71	2.71
4. „ 48	4.71	2.42

After heating for 1 hour.	Percentage of Coagulum.	
	Before digestion.	After digestion for 20 hours at 37° C.
1. To 50° C.	3.4	1.36
2. „ 55°	3.4	1.99
3. „ 60°	3.4	3.36
4. Unheated control expert. to 1-3	3.4	0.69
5. To 65° C.	2.72	2.57
6. Unheated control expert. to 5 .	2.72	0.87

the dry state, both in pressed yeast juice and permanent yeast, endotryptase naturally exhibits higher powers of resistance to heat. On the other hand, at low temperatures (3° to 7° C.)—as was shown by an experiment continued for fourteen days by Hahn and Geret—the digestion, though by no means entirely prevented, is so considerably delayed as to demonstrate the advantage of carrying out ordinary yeast fermentations at low temperatures, in view of the protection of the alcoholase (*see* p. 352, vol. ii.).

As in the case of other proteolytic enzymes, the influence of gases on the action of endotryptase seems to be very slight. Lack of oxygen was shown by the experiments of Geret and Hahn (I. and II.) to have no such favourable influence on the proteolysis of pressed yeast juice as was reported by Will (XXXV.) in the case of the living cells. On the contrary, the passage of air or oxygen was found rather to stimulate the digestion of protein; whilst the operation proceeded unhindered by the passage of a current of carbon dioxide or hydrogen. So far as carbon dioxide is concerned, this latter observation is also of practical importance to the fermentation process.

Weak antiseptics (chloroform, thymol, toluol, 0.2 per cent. salicylic acid, 0.1 per cent. formaldehyde) do not appear to have any influence on the action of endotryptase. On the other hand, powerful precipitants (3 per cent. phenol, 0.1 per cent. sublimate) naturally stop the digestion, which, however, contrary to the statements of Schär with regard to enzymatic action, is not restricted by 1 per cent. hydrocyanic acid. Even in concentrated solutions up to 5 per cent., according to T. Gromow (I.), of neutral salts, such as sodium, potassium and calcium chloride, stimulate proteolysis, whilst saturated solutions have a powerfully restrictive influence. Additions of 50 per cent. glycerine and saccharose also retard proteolysis considerably, and therefore preserve the alcoholase (*see* pp. 355, 356, vol. ii.), as well as the proteids. These discoveries of Geret and Hahn were supplemented by the observation of T. Gromow (I.), to the effect that, not only glycerine

and saccharose, but also mannitol, glucose, lactose, and glycocoll restrict the proteolysis of permanent yeast suspended in water. In the case of saccharose, this effect is already apparent on the addition of 5 per cent., whilst the proteolysis almost entirely ceases in presence of 35 per cent. Saccharose has a more powerfully restrictive action than glycerine or glycocoll in isotonic solutions; consequently, in this case the retarding effect must not be regarded as a purely physical process.

Pressed yeast juice, concentrated to one-third its original volume *in vacuo*, exhibited a considerably lessened autodigestion, the same effect being produced when permanent yeast was stirred up to a thick pap in water, instead of being suspended therein. Whether, as opined by T. Gromow (I.), the accumulation of metabolic products constituted the retarding factor has not yet been proved.

The influence of alcohol, which is always present, more or less, in yeast cultures, is also a matter of practical importance. Experiments by Geret and Hahn have demonstrated that the proteolysis of expressed yeast juice is slightly retarded by the presence of 5 per cent. alcohol, seriously so by 10–20 per cent. (this was confirmed by T. Gromow), and stopped by 30 per cent. alcohol. Hence, even in the advanced stages of fermentation of wine and beer, the complete suppression of the action of endotryptase by alcohol can hardly be expected to occur. According to Iwanoff (II.), the restriction of proteolysis during fermentation is effected, not by alcohol, the action of which (*see* p. 355, vol. ii.), does not become apparent until the concentration exceeds 4 per cent.; but by other volatile products, aldehydes and esters (fruit ethers). He states that the proteids are not decomposed by fermenting yeast in pure nutrient solutions; but in the ordinary complete nutrient solutions, in which all physiological processes are in full swing, even a small quantity of acid phosphates, as was found by Iwanoff himself, is able to entirely neutralise the restrictive action of the fermentation products on proteolysis, and in fact accelerate the latter.

Weak acids favour the action of endotryptase, the optimum effect being produced by the presence of 0.2 per cent. hydrochloric acid in pressed yeast juice, or by an equimolecular amount of sulphuric acid, whilst the same strength of acetic acid seems to act still more favourably. Boric acid (1 per cent.) or sodium borate (1 per cent.), does not retard proteolysis, whilst borax (*see* p. 179, vol. ii.) and all alkalis, even as weak as 0.1–0.2 per cent., diminish proteolysis considerably by the neutralisation of the pressed yeast juice.

The action of endotryptase is not confined merely to the nucleins of the yeast cell, but extends also to other proteids. Boullanger (I.) and Beijerinck (XXXI.) observed the digestion of casein by yeast; and Beijerinck obtained a similar result with glutin, albumen, and fibrin, whilst confirmation was furnished by Geret and Hahn (I. and II.) in respect of casein, glutin-casein, and albumen. In his experiments with pressed yeast, Schütz (I.) ascertained that endotryptase attacks yeast nuclein and gelatin the most, euglobin and serum albumen being far less powerfully decomposed, whilst in two cases out of three, pseudo-globulin was left intact.

With regard to the fission products resulting from the autodigestion of yeast, the older statements must be accepted with a certain degree of care, because it cannot always be safely deduced from them that the putrefactive influence of bacteria was precluded; whilst in some cases the method of experiment adopted was such as might induce the decomposition of the proteids. Thus, in their investigations on the chemical composition of yeast, Nägeli and Loew (II.) found 2 per cent. of peptones, which Loew subdivided into *a*-, *b*- and *c*-peptone (Meissner). This result, however, was obtained with an aqueous extract of yeast obtained by eleven successive prolonged boilings, so that Nägeli himself was obliged to admit that the boiling water might have produced hydrolysis. According to Geret and Hahn (I. and II.), albumoses and peptone are not

detected in expressed yeast juice digested at 37° C.; and in fact, even when albumoses and peptone are added to the juice, the biuret reaction soon disappears at a higher temperature. On the other hand, when the digestion is retarded by the temperature of the ice-chest, albumoses appear, these being chiefly deuteroalbumoses, whereas true peptones, in the sense indicated by Kühne, cannot be identified. Moreover, since F. Kutscher (III.) observed the occurrence of the biuret reaction for a period of 8–14 days, during the digestion of yeast with chloroform water—a process in which the enzyme only gradually issues from the cells and comes into action—the possibility of the formation of small quantities of albumoses during the protracted action of endotryptase must be admitted. Attention was also directed, at an early date, to other fission products occurring in the autodigestion of yeast. Thus, Liebig in 1868 referred to the occurrence of leucin during the autofermentation of yeast; the appearance of tyrosin among the fission products was observed by Béchamp in 1872; and both workers contemporaneously noticed a copious exudation of phosphoric acid from the yeast cell. Then followed Schützenberger's discovery of butalanin, alloxuric bases, carnin, sarkin, xanthin, and guanin (as well as tyrosin and leucin) in the aqueous extract from self-fermenting yeast. This worker regarded all these fission products as derivatives of albumen. The experiments of Kossel (III.) and Salkowski (II.) showed that the phosphoric acid and alloxuric bases should be regarded as fission products of the nuclein substances of the yeast cell, whereas the leucin and tyrosin probably originate in the decomposition of other proteids. This result is also confirmed by the autodigestion of expressed yeast juice. Geret and Hahn found that four-fifths to five-sixths of the phosphorus (mostly in the form of organic compounds) in expressed yeast juice is converted by this digestion into phosphoric acid, and that the greater part of the phosphorus can be identified as present in this form after digestion has been proceeding for an hour at 37° C. The amount of sulphuric acid, on the other hand, increases but slightly. The nitrogenous constituents of the expressed yeast juice undergo decomposition in such a manner that, at the close of the autodigestion process, about 30 per cent. of the nitrogen in the digestion products is in the form of bases, 70 per cent. being allocated to the amino acids, the proportion being the same as that in which these bodies are found in the fresh yeast juice freed from albumen. The xanthin bodies, which are present in far smaller amount (50–60 mgrms. per 100 c.c. of yeast juice) exhibit an interesting behaviour, inasmuch as, under normal conditions, they are still present in a latent form after the autodigestion of the yeast juice, and are only revealed by boiling with acids. This latent condition is probably due to the carbon dioxide appearing in the yeast juice in consequence of the fermentation.

The more intimate characterisation of the several fission products was effected by Kutscher, with the aid of Kossel's methods. In a recently published work, Kutscher and Lohmann (I.) give the following series of fission products, obtained by the autofermentation of beer yeast suspended in toluol water: guanin (abundant), adenin (abundant), xanthin (traces), hypoxanthin (traces), histidin, leucin, arginin, tyrosin, lysin, aspartic acid, glutamic acid (hitherto undetected), and ammonia (little). Shiga (I.) recently ascertained that the amount of xanthin increases continuously during the digestion of expressed yeast juice; but that guanin is decomposed, even when added in a fresh state; whilst adenin and hypoxanthin fluctuate. According to Shiga, the arginin is partially decomposed by an enzyme ("arginase") discovered in yeast juice, into urea and ornithin (α-δ-diaminovalerianic acid), a process that had been previously observed in the case of animal organs by Kossel and Dakin (I.).

Finally, Kutscher succeeded in also detecting cholin as a fission product of yeast

lecithin. This disposes of the hypothesis that cholin always originates entirely in the unfermented liquid (mash, wort, molasses, and must). The discovery of cholin is important, inasmuch as it is also capable of furnishing an explanation of the appearance of glycerine, namely, that, as mentioned on p. 367, vol. ii., the same may result from the decomposition of lecithin into fatty acids, cholin, and glycerophosphoric acid. Whether the other organic bases found in fermented liquids or their distillates (*see* p. 380, vol. ii.) owe their formation to the action of endotryptase must be regarded as at least doubtful. To this category belong the organic bases discovered in white wine by Brücke in 1855, the trimethylamine found in wine by E. Ludwig (I.) in 1867, the alkaloidal bases found in fermenting solutions of saccharose by Oser (I.), in beer by Lermer (I.), and in white wines by Guérin (I.), as well as the collidin found by Krämer and A. Pinner (I.), and the bases—which are regarded by Brand and Stoehr (II.) as probable derivatives of pyridin—observed by Schrötter (I.) in the fusel oil (*see* p. 376) of a molasses distillery. These discoveries cannot be attributed with certainty to the activity of the yeast. Other micro-organisms may have been concerned to some extent, and the products in question may have partly originated through the action of boiling heat on the residual proteids in the fermented liquids. The small amount of ammonia found to result from the pure autodigestion of yeast renders it at least improbable that any considerable production of volatile amine bases occurs, since most of these readily furnish ammonia. These fission products, however, really facilitate the more intimate characterisation of the proteolytic enzyme of yeast. As pointed out by Kutscher and Lohmann, they correspond exactly with those found by these authors in trypsin digestion. Salkowski, as well as Geret and Hahn, had already classed the enzyme as a tryptic enzyme, and regarded the identification of the monamino acids as sufficient for this characterisation. However, in spite of the identity of the fission products, it would not be advisable to assume that yeast endotryptase is the same as pancreatic tryptase, since two essential points of difference exist between them. In the first place, the action of yeast endotryptase is greatly facilitated by an acid reaction, and retarded by an alkaline reaction, in which respect its behaviour is the antithesis of that of pancreas tryptase; and, secondly, the autodigestion of expressed yeast juice yields substances that do not furnish the biuret reaction more than transitorily, if at all, the reaction quickly disappearing even when peptone and albumoses are added. Nevertheless, despite these differences, it must be maintained that we have here to deal with an enzyme belonging to the group of tryptases, more particularly because of the fission products obtained. For whether, as assumed by Lawrow (I.), such an extensive decomposition of the protein molecule can also be effected by peptases (the influence of which is favoured by an acid reaction) must still be considered doubtful, since in Lawrow's experiments with sliced pigs' stomachs it was impossible to preclude the occurrence of autolytic processes inseparable from the actual pepsin action.

The practically and theoretically important question of the conditions under which yeast endotryptase is formed and exerts its action is less easily answered. Will (XXXV.), who examined a large number of pure cultures of various species of yeast in wort-gelatin, and observed liquefaction (commencing in the path of the stab) within 18–20 days at 20° C., ascertained that the more rapidly liquefying species (*Mycoderma* and *Willia*) are in general more exacting as regards the supply of oxygen. When the inoculating material was mixed with the warmed (and thereby liquefied) gelatin, proteolysis was found to commence in 7–55 days, the time being proportional to the requirements of the species in respect of oxygen. On the basis of these and other observations, which need not be investigated more closely here, Will formed the conclusion that air

played a direct or indirect part in proteolysis by yeast, inasmuch as the presence of air either hinders the formation of a proteolytic enzyme, or destroys the same when already existing. According to Will, the liquefaction of gelatin is a function, not of moribund and decomposing cells, but of the normal cell, and is caused by a deficiency of nutriment, not merely a lack of dissolved substances in general, but of nitrogenous substances in particular, and of oxygen. Whereas Will regards dead yeast cells as merely an invariable concomitant of proteolysis, and denies the existence of any connection between proteolysis and the death of the cells, Beijerinck (XXXI.) assumes that the enzyme originates exclusively in cells that have perished in consequence of a scarcity of oxygen. Geret and Hahn, however, were able to show that lack of oxygen is not a decisive factor in the formation of the enzyme, inasmuch as they obtained an actively digestive expressed juice from fresh surface cultures of low-fermentation beer yeast, grown on wort agar-agar, under which conditions there was no lack of oxygen. Even fresh yeast cells, in all stages of growth, furnish leucin and other fission products, not only in the aqueous extract, but also in the fresh expressed juice, the proteid derivatives in the fresh cells being distributed among bases and amino acids in the same proportion as in the completely digested expressed juice. Hence, a proteolytic enzyme, or the zymogen of same, is present in yeast cells under all conditions; and, as opined by Kutscher, this enzyme probably exercises constructive functions, *i.e.*, it lessens the amount of the nitrogenous foodstuffs, prepared by the proteolytic enzymes of malt and diffused in the yeast cells, to such an extent that they can be utilised by the yeast cells for the elaboration of structural materials. Consequently the proteolytic enzyme is present as an intracellular inhabitant of every yeast cell.

The problem of the conditions under which the excretion of the enzyme occurs still remains to be discussed. That deprivation of oxygen does not form the decisive factor in this case also is most easily concluded from the circumstance that when the living cells are washed and lixiviated with distilled water, and are then left for twelve hours at the bottom of the vessel, this yeast, in a state of starvation as regards oxygen, cedes to the water an inverting enzyme, but not a proteolytic enzyme. On the other hand, the excretion of the enzyme and the process of autodigestion begin when the yeast is left without nitrogenous nutriment for some considerable time at a high temperature. In these circumstances, the corporeal substance of the starving yeast is attacked by the enzyme, probably the cell membrane first of all, the enzyme then acting destructively, as stated by Kutscher. It is, however, unnecessary to assume with Beijerinck that all the enzyme-generating cells have already perished, since it may be easily conceived that the death of a relatively small number of cells and the enzyme to which they have given rise leads to a modification of the nutrient medium or of the cell membrane of the surviving cells, whereby these latter are induced to excrete the enzyme. This may also perhaps explain the circumstance that Will could only find relatively few dead cells in the liquefied gelatin cultures.

These statements harmonise completely with the fact that Kutscher failed to detect the characteristic degradation products of yeast in pure lager beer. During the actual process of fermentation at a low temperature the number of moribund or pathologically modified yeast cells will presumably be very small, owing to the prevalence of the favourable environment, and consequently the amount of endotryptase or fission products passing out of the yeast cells and into the fermenting liquid will be strictly limited. The conditions may, however, be entirely different when the liquid is either left for a long time in contact with the sedimental yeast, *i.e.*, is not drawn off in good time, or else fermentation has been conducted at a higher temperature. In both cases it is not impossible for the

yeast to be acted on by the endotryptase (autodigestion), since both factors, prolonged contact of the liquid with the sedimental yeast and high temperature, favour the death of the yeast cells and the action of the endotryptase. Whether the increase in the proteid content of beer that has been fermented at a high temperature (20° C.), as was carried out by Hantke, has any connection with this subject is a matter that appears doubtful, in view of the energetic action of endotryptase on protein, which it quickly reduces to final products. On the other hand, one is bound to agree with K. Windisch (I.) in ascribing to this cause the peculiar flavour observed in low-fermentation beers that have been exposed to an unduly high temperature during primary fermentation (*see* p. 158, vol. ii.), and the flavour of digested expressed yeast juice is also indicative of the same thing.

Another important feature bearing on practice is the fact that alcoholase is destroyed by endotryptase (*see* p. 356, vol. ii.), so that in all cases where even a portion of the cells are dead, the fermentative power of the yeast may suffer, especially when the environment is unfavourable or the yeast is in a condition of famine, such as is particularly the case when the yeast is being watered or washed in the manufacture of pressed yeast. In this operation, in order to free the yeast from particles of grains or to classify it by sedimentation, it is left for a long time in contact with cold water ; and, as a matter of fact, the makers often find that the yeast is weakened, a result generally attributed to bacterial activity, to prevent which the yeast is treated with antiseptics. More probably, however, the loss of fermentative power may be explained by the action of endotryptase, for which the application of antiseptics affords no remedy. It will be a difficult matter to counteract this influence of endotryptase, though, certainly, the formation of endotryptase might be hindered, and the yeast consequently preserved, by improving the machinery so as to shorten the time occupied in washing. Again, it is not always right to assume that the rapid decomposition of the finished cakes of pressed yeast in the warm weather is due to the agency of bacteria, these latter very often only coming into action secondarily, after the way has been prepared for them by the death of the yeast cells and the action of the endotryptase.

§ 336.—Philothion.

J. de Rey-Pailhade (I.), in 1888, was the first to observe the faculty of an alcoholic extract of yeast to convert elementary sulphur into sulphuretted hydrogen. For this reason he named the active principle (enzyme) of the extract "philothion," which name he afterwards (VIII.) changed to "hydrogenase." An extract of this kind is easily prepared by treating yeast at ordinary temperature either with pure methylalcohol or with 86 per cent. ethylalcohol, the resulting yellow liquid being forced through a biscuit-ware filter in order to free it from cells. The extract becomes inactive on being kept at 70° C. for two hours. According to the same author (III.), the reducing power of this yeast enzyme is not restricted to sulphur, oxygen also coming within its sphere of activity, the yeast extract losing its powers when exposed to the air for a few days. The sensitivity of philothion, however, towards oxygen is not great, since it has been found by A. Wroblewski (I.) in an active condition in expressed yeast juice prepared without exclusion of air (*see* p. 343, vol. ii.). The negative results of G. Cossettini's (I.) attempt to confirm the reports of Rey-Pailhade seem attributable to the usual incapacity of enzymes to pass through the Chamberland filter (see p. 77, vol. i.) in certain circumstances.

Up to the present, philothion has not been isolated and prepared in a pure state ; and it is known and characterised solely from its reactions. It belongs to the group of the reductases, another member of which has been mentioned on p. 276, vol. ii., namely jacquemase, and from the whole of which it differs by its

characteristic action on free sulphur. Pozzi-Escott (II.) states that it is also able to convert phosphorus and selenium into their hydrogen compounds though it has no such action on tellurium or arsenic. According to Rey-Pailhade (IX.) free nitrous acid is destroyed by philothion very rapidly at 40° C., but more slowly at ordinary temperature. This power is crippled by dilute hydrochloric or sulphuric acid. In addition to being present in the cells of species of *Saccharomyces* and *Torula* (*see* p. 293, vol. ii.), this enzyme has been observed by Rey-Pailhade (II. and VI.) in various animal tissues and in germinating seeds. According to Pozzi-Escott (V.), it is retained by the first-named cells during the period of rapid reproduction, and is not diffused into the nutrient medium until fermentation has culminated in the latter.

Abelous and Ribaut (I.) denied the existence of philothion as such, and attempted to explain the above characteristic production of sulphuretted hydrogen by referring to the capacity of many proteids for readily parting with a portion of their sulphur in that state of combination. Pozzi-Escott (III.), however, demonstrated that the yeast extracts containing philothion lose their power of producing this gas in abundance, on being boiled; which was soon afterwards confirmed by Rey-Pailhade (X.); and that very energetic extracts are also able to reduce sulphites. According to Pozzi-Escott (VI.) this enzyme affords the yeast a means of defence against the poisonous action of sulphurous acid, a statement, however, in direct conflict with the just previously mentioned formation of that poison by philothion. Probably this last worker was correct in his opposition to the assumption of Gimel (I.), who regarded oxydase as the protective agent, and stated that yeast which has been habituated to large doses of sulphurous acid (*see* p. 329, vol. ii.) is able to produce larger quantities of oxydase than before habituation.

On the basis of his observation that methylene-blue is a more sensitive and rapid indicator than indigo-carmine, &c., for the detection of reducing enzymes, H. Hahn (IV.) investigated more closely the reducing power of expressed yeast juice. This power disappears within a few days when the yeast has been stored in the ice-chest in presence of toluol; and almost entirely vanishes on the juice being kept at 55°–60° C. The optimum temperature for reduction is 40° C., this agreeing with that (30°–40° C.) given by Pozzi-Escott (VIII.). The reducing power is lowered by diluting the yeast juice with water, whereas meat broth has a favourable influence. The reduction proceeds most rapidly in old yeast juice. The further observation of a certain parallelism between the fermentative and reducing action of the juice recalls the opinion held by J. Grüss on the part played by hydrogenase (philothion) in alcoholic fermentation (*see* p. 364, vol. ii.), namely, that it is the hydrogen temporarily formed during fermentation (and not philothion) that acts on free sulphur.

According to Pozzi-Escott (VIII.) the reduction is hindered most powerfully by salts with an acid reaction—mercury chloride and silver nitrate in particular, the nitrates being rather less injurious. Chloroform and acids retard, whereas alkalis stimulate the action.

More exhaustive investigations on the reducing power of yeasts and the real cause thereof will not only increase the sum of our knowledge on the theory of enzymes, but may be of practical utility to the fermentation industry. The appearance of sulphurous acid and sulphuretted hydrogen in fermenting musts, worts, and washes, and their occurrence in wine, beer, and spirits, were already known to fermentation technologists and analytical chemists at a time before any one had begun to speak of reductases. On this point the reader is referred to p. 170, vol. ii., and to the recent publications of Frew (I.), A. Ostwalder (I. and II.), R. Schander (IV.), W. Seifert (V. and VI.), H. Will and H. Wanderscheck (I.), W. Windisch (VII.) and J. Wortmann (XXI.)

BIBLIOGRAPHY.

As will be readily seen, the following bibliography not only gives the original publications but also references to the more widely distributed and easily accessible journals, as many readers may wish to go deeper into one or more branches of the subject than they could do within the limits of *Technical Mycology*. These references, on account of their frequently critical importance, will often be useful even to those who have the original papers at their command. The authors of *Technical Mycology* cannot claim to belong to this category. The titles of the most important journals are abbreviated in order to economise space, and the following list will explain them:

CONTRACTIONS.

A. f. Hygiene	=	Archiv für Hygiene.
Abt.	=	Abteilung = Section.
Ann. Inst. Past.	=	Annales de l'Institut Pasteur.
Ber. d. D. Bot. Ges.	=	Berichte der Deutschen Botan. Gesellschaft.
Ber. d. D. Chem. Ges.	=	Berichte d. Deutschen Chemischen Gesellschaft.
Bot. Ztg.	=	Botanische Zeitung.
Ch. C.	=	Chemisches Centralblatt.
C. f. B.	=	Centralblatt für Bakteriologie.
C. R.	=	Comptes rendus de l'Académie des Sciences, Paris.
K. J.	=	Koch's Jahresbericht über die Fortschritte in der Lehre von Gärungsorganismen.
Pflüger's Archiv	=	Archiv für die gesamte Physiologie (Pflüger).
Ref.	=	Referred to in.
Vers.-Stat.	=	Die landwirthschaftl. Versuchs-Stationen (Nobbe).
W. f. Br.	=	Wochenschrift für Brauerei.
Z. f. Hygiene	=	Zeitschrift für Hygiene und Infektionskrankheiten.
Z. f. physiolog. Chemie	=	Zeitschrift für physiolog. Chemie.
Z. f. Spiritusind.	=	Zeitschrift für Spiritusindustrie.
Z. g. Br.	=	Zeitschrift für das gesamte Brauwesen.

ABBA, Francesco,

I. Riconoscimento dell' arsenico in una farina per mezzo del Penicillium brevicaule.—Rivista d'Igiene e Sanità publica. 1893. p. 831.—Hygien. Rundschau. 1894. IV. 325.—Ch. C. 1894. II. 112.

II. Über die Feinheit der biologischen Methode beim Nachweis des Arseniks.—C. f. B. 2. Abt. 1898. IV. 806.—Ch. C. 1898. II. 1281.

ABEL, Rudolf,

I. Über die Brauchbarkeit der Schild'schen Formalinprobe zur Differentialdiagnose des Typhusbacillus.—C. f. B. 1894. XVI. 1041.—Ch. C. 1895. I. 277.

ABEL, Rudolf, und BUTTENBERG, Paul,

I. Über die Einwirkung von Schimmelpilzen auf Arsen und seine Verbindungen.—Z. f. Hygiene. 1899. XXXII. 449.—C. f. B. 2. Abt. 1900. VI. 187.—Ch. C. 1900. I. 428.

ABEL, Rudolf, und DRÄER, Arthur,

I. Das Hühnerei als Kulturmedium für Choleravibrionen.—Z. f. Hygiene. 1895. XIX. 61.—Ch. C. 1895. I. 697.

ABELES, Hans,

I. Zur Frage der alkoholischen Gärung ohne Hefezellen.—Ber. d. D. Chem. Ges. 1898. XXXI. 2261.—C. f. B. 2. Abt. 1899. V. 40.—Ch. C. 1898. II. 894. K. J. IX. 315.

ABELOUS, A., et H. RIBAUT,

I. C. R. 1903. CXXXVII. 95, 3268.

ABERSON, J. H.,

See GILTAY und ABERSON.

ADAMETZ, Leopold,

I. Die Bakterien der Trink- und Nutzwässer.—Mitteilungen der österr. Versuchsstation für Brauerei u. Mälzerei in Wien. I. Heft. p. 19.

II. Die Bakterien normaler und abnormaler Milch.—Österr. Monatsschrift f. Tierheilkunde und Tierzucht. 1890. V. 1.—C. f. B. 1890. VIII. 109.

III. Über die Ursachen und Erreger der abnormalen Reifungsvorgänge beim Käse.—Milchzeitung. 1891. XX. 237. 1892. XXI. 205.—K. J. II. 196; III. 184.

IV. Über einen Erreger der schleimigen Milch, Bacillus lactis viscosus.—Milchzeitung. 1889. XVIII. 941.—C. f. B. 1890. VII. 767.

V. Untersuchungen über Bacillus lactis viscosus, einen weit verbreiteten milchwirtschaftlichen Schädling. — Landw. Jahrbücher. 1891. XX. 185.—C. f. B. 1891. IX. 698.—K. J. II. 182.

VI. Bakteriologische Untersuchungen über den Reifungsprozess der Käse.—Landw. Jahrbücher. 1889. XVIII. 227.—C. f. B. 1889. VI. 78.

VII. Über Micrococcus Sornthalit.—C. f. B. 2. Abt. 1895. I. 465.—K. J. VI. 248.

VIII. Kritische Bemerkungen über F. Baumann's Beiträge zur Erforschung der Käsereifung.—Deutsche Molkerei-Zeitung. 1893. No. 16.—K. J. IV. 213.

IX. Untersuchungen über die niederen Pilze der Ackerkrume. Dissert. Leipzig. 1886.—C. f. B. 1887. I. 8.

X. Saccharomyces lactis, eine neue Milchzucker vergärende Hefeart.—C. f. B. 1. Abt. 1889. V. 116.

XI. C. f. B. 1889. V. 110.

XII. Über die Ursachen und Erreger abnormalen Reifungsvorgänge beim Käse. Bremen. 1893.

XIII. Untersuchungen ü. d. niederen Pilze d. Ackerkrume. Dissert. Leipzig. 1886. p. 39.

Adametz, L., und Wilckens, Martin,

I. Milchwirtschaftliche Untersuchungen, etc.—Landwirtsch. Jahrbücher. 1892. XXI. 131.—C. f. B. 1892. XII. 98.—K. J. III. 178.

Aderhold, Rudolf,

I. Die Morphologie der deutschen Saccharomyces ellipsoideus-Arten.—Landw. Jahrbücher. 1894. XXIII. 587.—C. f. B. 2. Abt. 1895. I. 410.—K. J. V. 159.

II. Über den Einfluss der Kohlensäure auf die normale Gärung störende Organismen, mit Bemerkungen über die Konservierung des Weines. — Mitteilungen über Weinbau und Kellerwirtschaft. 1892. IV. 132.

III. Studien über eine gegenwärtig in Mombach bei Mainz herrschende Krankheit der Aprikosenbäume und über die Erscheinungen der Blattranddürre. — Landw. Jahrb. 1893. XXII. 435.—Bot. Ztg. 1893. II. 267.

IV. Landw. Jahrbücher. 1899. XXVIII. 69.

V. C. f. B. 2. Abt. 1899. V. 511.

Aeby, J. H.,

I. Beitrag zur Frage der Stickstoffernährung der Pflanzen.—Vers.-Stat. 1896. XLVI. 409.—Ch. C. 1896. 818.—K. J. VII. 206.

Ahlburg,

I. *See* Korschelt, II.

Ahrens, Felix B.,

I. Ein Beitrag zur zellenfreien Gärung.—Zeitschr. f. angewandte Chemie. 1900. XIV. 483.—C. f. B. 2. Abt. 1900. VI. 744.—Ch. C. 1900. II. 52.—K. J. XI. 368.

Albert, Friedrich,

I. Die Umfrage über die Braunheuherstellung.—Mitteil. d. deutsch. Landwirtsch.-Gesellsch. pro 1893–94. II. 14.

II. Untersuchungen über Grünpressfutter.—Jahrb. d. deutsch. Landwirtsch.-Gesellsch. Bd. VI. Tl. I. p. 149. Berlin. 1891.—(Gives the principal literature thereon.)

Albert, Robert,

I. W. f. Brauerei. 1899. XVI. 485.

Albert, Rudolf,

I. Einfacher Versuch zur Veranschaulichung der Zymase-Wirkung. — Ber. d. D. Chem. Ges. 1900. XXXIII. 3775.—C. f. B. 2. Abt. 1901. VII. 473.—Ch. C. 1900. I. 469.—K. J. XI. 364.

II. Über künstliche Anreicherung der Hefe an Zymase.—Ber. d. D. Chem. Ges. 1899. XXXII. 2372.—C. f. B. 2. Abt. 1900. VI. 89.—Ch. C. 1899. II. 915.—K. J. X. 312.

III. Erfahrungen bei der Herstellung von Hefepresssaft aus untergäriger Bierhefe der Versuchs und Lehrbrauerei zu Berlin.—W. f. Br. 1899. XVI. 485. K. J. X. 310.

See also Albert u. Buchner.

Albert, R., und Buchner, Ed.,

I. Hefepresssaft und Fällungsmittel.—Ber. d. D. Chem. Ges. 1900. XXXIII. 266, 971.—W. f. Br. 1900. XVII. 49, 189.—C. f. B. 2. Abt. 1900. VI. 373, 536.—Ch. C. 1900. I. 677, 1034.—K. J. XI. 365, 367.

II. Ber. d. D. Chem. Ges. 1899. XXXII. 2372.

III. *Ibid.* 1900. XXXIII. 266; 971.

Albert, R., Buchner, E., u. Rapp, R.,

I. Ber. d. D. Chem. Ges. 1902. XXXV. 2376.

Ali-Cohen, Ch. H.,

I. Eigenbewegung bei Mikrokokken.—C. f. B. 1889. VI. 33.

II. Die Chemotaxis als Hilfsmittel der bakteriologischen Forschung.—C. f. B. 1890. VIII. 161.—K. J. I. 8.

Allik, A. K.,

I. Die chemische Analyse des Kumys. Dissert. Dorpat. 1895.—K. J. VI. 223.

ALTEHOEFER,
I. Über die Desinfectionskraft von Wasserstoffsuperoxyd auf Wasser.—C. f. B. 1890. VIII. 129.—(Also gives the literature prior to date.)

ALTENBURG,
I. Quoted by KOBERT, III.

ALTMANN, Richard,
I. Über Nucleinsäuren.—Archiv d. Anatomie u. Physiologie. 1889. Physiologie. Abt. S. 524.—Ch. C. 1890. I. 126.

ALVAREZ,
I. Sur un nouveau microbe, déterminant la fermentation indigotique et la production de l'indigo bleu.—C. R. 1887. CV. 286.—C. f. B. 1887. II. 441.

AMAND, A.,
I. La Cellule. 1902. XX. 225.
II. *Ibid.* 1904. XXI. 329.

AMANN, P.,
I. Pleochroismus gefärbter Bakterienzellen.—C. f. B. 1893. XIII. 775.—K. J. IV. 54.

AMTHOR, Karl,
I. Über den Saccharomyces apiculatus.—Z. f. physiolog. Chemie. 1888. XII. 558; reprinted from Z. g. Br. 1888. XI. 370.—Ch. C. 1888. p. 1120.
II. Beobachtungen über den Saccharomyces apiculatus.—Chem.-Zeitg. 1891. p 670.—Ch. C. 1891. I. 1077.—K. J. II. 141.
III. Über Hefeweine und den Ammoniak-Gehalt in Most und Wein.—Zeitschr. f. angewandte Chemie. 1890. p. 27.—Ch. C. 1890. I. 237.—K. J. I. 66.
IV. Z. f. physiolog. Chemie. 1888. XII. 64.
V. Z. f. angew. Chemie. 1892. 319.

ANCLÉS, Louis Edgar,
I. Das Konservieren der Nahrungs- und Genussmittel. Wien. 1895. Hartleben.

ANDRÉ, G.,
See BERTHELOT et ANDRÉ.

ANDREASCH, F.,
I. Gärungserscheinungen in Gerbbrühen.—Der Gerber. 1895. p. 193. 1896. S. 3.—Zeitschrift f. angewandte Chemie. 1896. p. 216.

ANDRLIK, K.,
I. Das Verhalten der Raffinose bei der Vergärung von Melasse.—Zeitschrift f. Zucker-Industrie in Böhmen. 1898. XXIII. I.—Ch. C. 1898. II. 1273.

ANSAI,
I. Mitt. d. mediz. Gesellsch. zu Tokio. 1904. XVI. No. 6, p. 1.

APPERT, M.,
I. Le livre de tous les ménages ou l'art de conserver, pendant plusieurs années, toutes les substances animales et végétales. Quatrième édition. Paris. 1831.

APOSTOLI et LAQUERRIÈRE,
I. De l'action polaire positive du courant galvanique constant sur les microbes et en particulier sur la bactéridie charbonneuse.—C. R. 1890. CX. 918.—K. J. I. 44.

ARAKI, T.,
I. Über das Chitosan.—Z. f. physiolog. Chemie. 1895. XX. 498. (It gives the antecedent literature relating to the formulæ for Chitin.)—Ch. C. 1895. I. 1179.

ARATA,
I. Über die Veränderungen, denen die flüchtigen Säuren der Butter beim Ranzigwerden derselben unterworfen sind, und über die Wirkung der ranzigen Butter auf den Organismus.—Annalen des Inst. f. Experimentalhygiene in Rom. 1893.—K. J. IV. 181.

ARCANGELI, G.,
I. Sulla fermentazione panaria. — Atti della Società toscana di scienze naturali residente in Pisa. 1888. IX. 1.—C. f. B. 1888. III. 717.—Ch. C. 1888. 974.

ARCHANGELSKI,
I. Über Milzbrand.—Centralblatt f. d. med. Wissenschaften. 1883. 257.

ARCHLEB, Josef,
I. Uber den Einflüss der Konzentration der Nährflüssigkeiten auf die Vermehrung der Alkoholfermente und den Vergärungsgrad. 1887. Neustadt a. d. Mettau.—Z. f. Spiritusind. 1888. XI. 243.

ARLOING, S.,
I. De l'influence des filtres minéraux sur les liquides contenant des substances d'origine microbienne.—C. R. 1892. CXIV. 1455.—C. f. B. 1892. XII. 882.—K. J. III. 21.

ARNHEIM, J., u. ROSENBAUM, J.,
I. Z. f. physiolog. Chemie. 1903. XI. 220.

ARONSON, Hans,
I. Uber die antiseptischen Eigenschaften des Formaldehydes.—Berliner klinische Wochenschrift. 1892. XXIX. No. 30.—Ch. C. 1892. II. 579.

ARSONVAL, A. d',
I. La pression osmotique et son rôle de défense contre le froid dans la cellule vivante.—C. R. 1901. CXXXIII. 84.—Ch. C. 1901. II. 433.

ARSONVAL, A. d', et CHARRIN, A.,
I. Electricité et microbes. — Comptes rendus de la société de biologie. Paris. 1893, p. 467.—K. J. IV. 114.
II. Concurrence vitale entre le bacille pyocyanique et la levure de bière.—*Ibid.*, p. 70 *et seq.*—K. J. IV. 116 u. f.

ARTARI, A.,
I. Abhandlungen d. Nat. Ges. zu Halle. 1897. XXI. 113.

ARTARI, Alexander,
I. Über einen im Safte der Zuckerfabriken in Gemeinschaft mit Leuconostoc schädlich auftretenden, den Zucker zu Alkohol und Säuren vergärenden Saccharomyces (S. Zopfii). — Abhandlungen d. Naturforschenden Ges. zu Halle. 1897. XXI. 113.—C. f. B. 2. Abt. 1897. III. 529.—K. J. VIII. 101.

ARTHUS, M., et PAGÈS, C.,
I. Recherches sur l'action du lab et la coagulation du lait dans l'estomac et ailleurs.—Arch. de physiologie. Vème série. 1890. II. 331.—K. J. I. 173.
II. Sur le labferment de la digestion du lait. Arch. de physiologie. Vème série. 1890. II. 540.—K. J. I. 174.

ARUSTAMOFF, M.,
I. Über die Natur des Fischgiftes.—C. f. B. 1891. X. 113.

ASBÓTH, Alexander von,
I. Über das Vorkommen von Pyridin in manchen Amylalkoholen. — Chemiker-Zeitg. 1889. XIII. 871.—Ch. C. 1889. II. 423.

ASCOLI, Alberto,
I. Über die Plasminsäure.—Z. fur physiolog. Chemie. 1899. XXVIII. 426. —Ch. C. 1900. I. 43.
II. Über den Phosphor der Nucleinstoffe.—*Ibid.* 1900. XXXI. 156.—Ch. C. 1901. I. 127.
III. Über ein neues Spaltungs produkt des Hefenucleins.—*Ibid.* 1900. XXXI. 161. —Ch. C. 1901. I. 127.

ASO,
I. Quoted by KOZAI, II.

ASTIER, Charles Benoit,
I. Rapport des expériences faites sur le sirop et le sucre de raisin.—Annales de Chimie. 1813. LXXXVII. 27.
II. Sur la transmutation du sirop de raisin en vin. — Journal des propriétaires ruraux pour le midi de la France. XVII.

ATKINSON, R. W.,
I. Sur la diastase du Koji.—Moniteur scientifique de Quesneville. 1882, p. 7.

AUBRY, Louis,
I. Das Antinonnin im Dienste der Bierbrauerei.—Z. g. Br. 1893. XVI. 141.
II. Ein Beitrag zur Klärung und Richtigstellung der Ansichten über reine Hefe. —*Ibid.* 1885. VIII. 133.
III. Z. g. Br. 1894. XVII. 1.
IV. *Ibid.* 1897. XX. 631.

AXENFELD, D.,
I. Centralbl. f. Physiologie. 1903. XVII. 268.

BABES, Victor,
I. Über isoliert färbbare Anteile von Bakterien.—Z. f. Hygiene. 1888. IV. 173.

BABO, A. Freiherr von, und MACH, Edmund,
I. Handbuch des Weinbaues und der Kellerwirtschaft. 3rd Ed. I. Band. Weinbau. 22*s.*; II. Band: Kellerwirtschaft. 24*s.* Berlin, P. Parey. 1896.

BACH, Karl,
I. Die Verarbeitung und Konservierung des Obstes und der Gemüse. Stuttgart, Ulmer. Bound, 3*s.*

BACHMANN, Hans,
See BACHMANN, Johann.

BACHMANN, Johann,
I. Einfluss der äusseren Bedingungen auf die Sporenbildung von Thamnidium elegans Link.—Bot. Ztg. 1895. 1. Abt. LIII. 107.
II. Mortierella van Tieghemi nov. spec. —Jahrb. für wissenschaftl. Botanik. 1899. XXXIV. 279.

BAEYER, A.,
I. Ber. d. D. Chem. Ges. 1870. III. 63.

BAGINSKY, Adolf,
I. Rote Milch. — Deutsche Medizinal-Zeitung 1889. No. 9.—C. f. B. 1. Abt. 1889. V. 448.
II. Zur Biologie der normalen Milchkotbakterien.—Z. f. physiolog. Chemie. 1888. XII. 434. 1889. XIII. 352.—C. f. B. 1. Abt. 1888. IV. 201. 1889. VI. 16.—Ch. C. 1888. S. 975. 1889. I. 522.
III. Zum Grotenfelt'schen Bacillus der roten Milch.—Deutsche mediz. Wochenschrift. 1889. XV. 212.—C. f. B. 1. Abt. 1889. VI. 137.—Ch. C. 1889. I. 691.
IV. Über das Vorkommen von Xanthin, Guanin und Hypoxanthin.—Z. f. physiolog. Chemie. 1884. VIII. 395.

BAIL, O.,
I. C. f. B. 2. Abt. 1902. VIII. 567.

BAIL, Theodor,
I. Über Hefe.—Reprinted from Kunst- und Gewerbeblatt d. polytechn. Vereins f. Bayern. 1857. p. 11.
II. Über die Entstehung der Hefe.—Journal f. prakt. Chemie. 1867. CI. 47.
III. Flora. 1857. XL. 417.

BAINIER,
I. Bull. Soc. Mycol. de France. 1905. XXI. 126.

BALCKE, Julius,
I. Bakterientrübung im Bier.—W. f. Br. 1884. I. 181.

BALARD,
I. Ann. Chem. et de phys. 1844. (3.) XII. 294.

BALLAND,
I. Observations sur les farines.—C. R. 1894. CXIX. 565.—Ch. C. 1894. II. 895.

BALLAND et MASSON,
I. Sur la stérilisation du pain et du biscuit sortant du four.—C. R. 1893. CXVII. 797.—Ch. C. 1894. I. 178.

BALLARD, Antoine Jérome,
I. Sur une altération spontanée de certains vins.—C. R. 1861. LIII. 1226.

BALLING, C. J. N.,
I. Die Gärungschemie. 3rd Ed. 1865. II. 239.

BALOGH, N. von,
See FUNK und BALOGH.

BAMBERGER, E., und A. EINHORN,
I. Ber. d. D. Chem. Ges. 1897. XXX. 224.

BANG, Ivar,
I. Die Guanylsäure der Pankreasdrüse und deren Spaltungsprodukte.—Z. für physiolog. Chemie. 1898. XXVI. 133. —Ch. C. 1898. II. 1210.

BANNING,
I. C. f. B. 2. Abt. 1902. VIII. 395.

BARBA, G.,
See KAYSER et BARBA.

BARBET,
I. Bull. de l'assoc. des Chimistes. 1900. XIX. 1107.

BARFOED, C.,
I. J. f. prakt. Chem. 1873. VI. 334.

BARKER,
I. Ann. of Botany. 1903. XVII. 187.

BARKER, B. T. P.,
I. Proc. Roy. Soc. 1901. LXVIII.—Phil. Trans. 1901. CXCIV. 467.

BARKER, P.,
I. Ann. of Botany. 1900. XIV. 215.

BARTH, G.,
I. Z. g. Br. 1900. XXIII. L. 909.
II. Ber. d. D. Chem. Ges. 1878. XI. 474.

BARTEL, A.,
See SCHROEDER und BARTEL.

BARTH, Max,
I. Die Obstweinbereitung mit besonderer Berücksichtigung der Beerenobstweine. —4. Ed. Stuttgart. 1897. E. Ulmer. 1*s.* 4*d.*

BARY, Anton de,
I. Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bakterien. —Leipzig. 1884. Engelmann.
II. Über einige Sclerotinien und Sclerotienkrankheiten. — Bot. Ztg. 1886. XXXIV. 377.
III. Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. 1866.
IV. Uber die Fruchtentwicklung der Ascomyceten. 1863. Leipzig.
V. Vorlesungen über Bakterien. 2. Ed. 1887. Leipzig.
VI. Beiträge zur Morphologie und Physiologie der Pilze. I. Series. Frankfurt a. M. 1864.
VII. *Ibid.*, II. Series. 1866.
VIII. *Ibid.*, 3rd Series. 1870. 2. Abt. 18.
IX. Bot. Ztg. 1854. 425.

BASENAU, Fritz,
I. Über die Ausscheidungen von Bakterien durch die thätige Milchdrüse und über die sogen. baktericiden Eigenschaften der Milch.—A. f. Hygiene. 1895. XXIII. 44.—Ch. C. 1895. I. 1070.

BASILE, G.,
I. Mannitgärung der Weine in Sicilien.—Staz. sperim. agrar. ital. 1894. XXVI. 451.—Ch. C. 1894. II. 498.—K. J. V. 194.

BAU, Arminius,
I. Über die scheinbare Zunahme des Dextringehaltes in Bierwürzen während der Gärung, sowie über die Bestimmung der Dextrose und des Dextrins in denselben.—W. f. Br. 1890. VII. 1069. —C. f. B. 1891. IX. 99.—Ch. C. 1890. II. 863.—K. J. I. 69.
II. Über die Zusammensetzung der Bierwürzen in Bezug auf Kohlehydrate.—W. f. Br. 1891. VIII. 1.—Ch. C. 1891. I. 513.—K. J. II. 139.
III. Die Bestimmung von Maltose, Dextrose und Dextrin in Bierwürze und in Bier mittelst Reinkulturen von Gärungsorganismen.—C. f. B. 1891. IX. 825. —Ch. C. 1891. II. 92.—K. J. II. 141.
IV. Zur quantitativen Bestimmung der Isomaltose.—W. f. Br. 1892. IX. 1421. —Ch. C. 1893. I. 233, 960.—K. J. III. 127.
V. Über die Verwendung der Hefe zur quantitativen Bestimmung gärfähiger Substanzen. — Chemikerzeitg. 1893. XVII. 392.—Ch. C. 1893. I. 802.—K. J. IV. 135.
VI. Der Sammelbegriff Saccharomyces cerevisiae.—W. f. Br. 1894. XI. 1366. —C. f. B. 2 Abt. 1895. I. 88.—Ch. C. 1894. II. 1067.
VII. Über den Nachweis von Unterhefe in obergäriger Presshefe.—Z. f. Spiritusind. 1894. XVII. 374.—Ch. C. 1895. I. 91.—K. J. V. 159.
VIII. Prüfung der Presshefe auf eine Beimengung von Unterhefe. — Z. f. Spiritusind. 1895. XVIII. 372.—C. f. B. 2. Abt. 1896. II. 98.—K. J. VI. 166.
IX. Über Schwergärigkeit der Melassen. —Z. f. Spiritusind. 1894. XVII.—K. J. V. 158.
X. Über Raffinose oder Melitriose und über ihre Abwesenheit im Bier.—W. f. Br. 1894. XI. 1439.—K. J. V. 141.
XI. Über das Verhalten der Oberhefe gegenüber der Isomaltose und der Raffinose.—W. f. Br. 1894. XI. 113. —K. J. V. 140.
XII. Über ein neues Enzym der Hefe. —Chemikerzeitg. 1895. XIX. 1873. —C. f. B. 2. Abt. 1895. I. 887.—Ch. C. 1895. II. 1049.—K. J. VI. 319.
XIII. Über Melitriose und deren quantitative Bestimmung.—Chemikerzeitg. 1894. XVIII. 1794. (Also gives the history of this sugar.) Ch. C. 1895. I. 27.
XIV. Über Melibiose. — Chemikerzeitg. 1897. XXI. 186.—Ch. C. 1897. I. 744.
XV. Beiträge zur Vergärbarkeit und zur analytischen Verwertung der Melitriose.—W. f. Br. 1898. XV. 389.—Ch. C. 1898. II. 682.
XVI. Über krystallisierte Melibiose.—W. f. Br. 1899. XVI. 397.—Ch. C. 1899. II. 526, 644.
XVII. Über die Vergärbarkeit der Galactose.—Z. f. Spiritusind. 1896. XIX. 303.—C. f. B. 2. Abt. 1896. II. 653. —K. J. VII. 97.

XVIII. Über Gärversuche mit Trehalose. —W. f. Br. 1899. XVI. 305.—C. f. B. 2. Abt. 1899. II 871.—Ch. C. 1899. II. 130.
XIX. Über Obergärung und Reinzucht. —W. f. Br. 1892. IX. 1057.—K. J. III. 158.
XX. W. f. Brauerei. 1891. VIII. 592.
XXI. *Ibid.* 1892. IX. 1185.
XXII. Z. f. Spiritusind. 1904. XXVII. 317.
XXIII. Z. f. Spiritusind. 1896. XIX. 303.
XXIV. Chem. Zeitg. 1892. XVI. 143. —W. f. Brauerei. 1892. XIX. 193.
XXV. W. f. Brauerei. 1902. XIX. 44.
XXVI. W. f. Brauerei. 1903. XX. 560.
XXVII. Beitr. d. Vereins a. d. Rüben-Zuckerindustrie. 1904. LIV. 481.
XXVIII. Chem. Beitr. 1897. XXI. 185.
XXIX. Z. f. Spiritusind. 1898. XXI. 241.
XXX. W. f. Brauerei. 1900. XVII. 698.
XXXI. Chem. Ztg. 1892. XVI. 1473. 1893. XVII. 392.
XXXII. W. f. Brauerei. 1895. XII. 431.

Bauer,
I. Abgeänderter Sterilisator. — Z. g. Br. 1895. XVIII. 85.—K. J. VI. 11.
II. J. f. prakt. Chem. 1884. XXX. 283.

Bauer, Emil,
I. Über die Vergärbarkeit der Melasse.— Z. f. Spiritusind. 1894. XVII. No. 14. —K. J. V. 157.
II. Ungar. Patent No. 12,075, Aug. 12, 1898.
III. Untersuchungen über Gärung, Ernährung und Vermehrung von Hefe.—Z. f. Spiritusind. 1901. XXIV. 309.—C. f. B. 2. Abt. 1902. VIII. 650.

Bauer, H.,
See C. Pohl und H. Bauer.

Bauke, H.,
I. Beiträge zur Kenntnis der Pykniden. I. —Nova acta academiae caesareae Leopoldino-Carolinae germanicae naturae curiosorum. 1876. XXXVIII.
II. Zur Entwicklungsgeschichte der Ascomyceten.—Bot. Ztg. 1877. XXXV. 313.

Baumann, E.,
I. Über die Bildung von Phenol bei der Fäulnis.—Ber. d. D. Chem. Ges. 1877. X. 685.—Ch. C. 1877. 391.
II. Bildung der Hydroparacumarsäure aus Tyrosin.—Ber. d. D. Chem. Ges. 1879. XII. 1450.—Ch. C. 1879. 599.
See also Baumann und Brieger.

Baumann, E., und Brieger, L.,
I. Über die Entstehung von Kresolen bei der Fäulnis.—Z. f. physiolog. Chemie. 1879. III. 149.—Ch. C. 1879. 329.

Baumann, Fritz,
I. Beiträge zur Erforschung der Käsereifung.—Landw. Versuchsstationen. 1893. XXXXII. 181.—C. f. B. 1893. XIV. 494.—K. J. IV. 209.

Baumgarten, P.,
I. Lehrbuch der pathologischen Mykologie. 2 Bände. Braunschweig. H. Bruhn. 1890.

Bay, J. Chr.,
I. Tubercular Infectiousness of Milk.— Annual Report of the Iowa State Dairy Commissioner. Des Moines. 1896.—K. J. VII. 166.
II. The American Naturalist. 1893. XXVII. 693.
III. C. f. B. 2. Abt. 1896. II. 259.
IV. Ber. d. D. Bot. Ges. 1894. XII. 90.

Béchamp, A.,
I. Les Mikrozymas dans leurs rapports avec l'hétérogenie, l'histogénie, la physiologie et la pathologie. 992 pp.— Paris 1883.
II. Sur la viscose ou substance gommeuse de la fermentation visqueuse : équation de cette fermentation.—C. R. 1881. LXXXXIII. 78.—Z. g. Br. 1881. IV. 482.
III. De la réduction des nitrates et des sulfates dans certaines fermentations.— C. R. 1868. LXVI. 547.—Ch. C. 1868. p. 942.
IV. Gärung der Apfelsäure.—Bulletin de la Société chimique de Paris. 1894. XI. 466.—Ch. C. 1894. II. 100.
V. Gärung der Bernsteinsäure und der Brenzweinsäure.—Bulletin de la Société chimique de Paris. 1894. XI. 418.— Ch. C. 1894. II. 47.
VI. Nouvelle méthode d'incinération des matières végétales et animales ; application au dosage des éléments minéraux de la levure. C. R. 1871. LXXIII. 337.
VII. Sur l'épuisement physiologique et la vitalité de la levure de bière.—C. R. 1865. LXI. 689.
VIII. Sur la cause de la fermentation alcoolique par la levure de bière et sur la formation de la leucine et de la tyrosine dans cette fermentation.—C. R. 1872. LXXIV. 184.
IX. Recherches sur la nature et l'origine des ferments.—Annales de chimie et de physique. 4e sér. 1871. XXIII. 442.
X. Nouvelles recherches sur l'épuisement physiologique de la levure de bière et remarques à l'occasion d'une récente Communication de M. Schützenberger sur le même sujet. — C. R. 1874. LXXVIII. 645.
XI. Faits pour servir à l'histoire de la levure de bière et de la fermentation alcoolique.—C. R. 1879. LXXXVIII. 866.—Z. g. Br. 1879. II. 356.
XII. C. R. 1864. LIX. 496.
XIII. C. R. 1872. LXV. 1036.
XIV. C. R. 1863. LVI. 969, 1184.

Bechhold, Jakob,
I. Untersuchungen an dem Klärbeckenschlamm zu Frankfurt a. M.—Z. f. angew. Chemie. 1899. p. 849.—Ch. C. 1899. II. 726.

BECK, M.,
See PROSKAUER und BECK.

BECKER, C.,
I. Über Schichtung und Färbbarkeit der Membran der Hefezellen.—Z. g. Br. 1899. XXII. 597.—C. f. B. 2. Abt. 1900. VI. 24.

BECKER, H.,
I. Neuerungen auf dem Gebiete des Gärungswesens.—Z. g. Br. 1897. XX. 437.
See also POPP und BECKER.

BEHR, P.,
I. Über eine nicht mehr farbstoffbildende Rasse des Bacillus der blauen Milch.—C. f. B. 1890. VIII. 485.—K. J. I. 40.

BEHREND, Paul,
I. Beiträge zur Chemie des Obstweines und des Obstes., p. 57.—Hohenheim 1892.—Ch. C. 1893. I. 327.

BEHREND, P., u. MORGAN, A.,
I. Über die Veränderungen, welche die stickstoffhaltigen Verbindungen der süssen Maische durch die Gärung erfahren.—Vers.-Stat. 1880. XXIV. 171.

BEHRENS, Johann,
I. Der Ursprung des Trimethylamins im Hopfen und die Selbsterhitzung desselben.—Karlsruhe. 1894. O. Nemnich.—K. J. V. 76.
II. Weitere Beiträge zur Kenntnis der Tabakpflanze.—VII. Die Fermentation.—Vers.-Stat. 1894. XXXXIII. 293.—Ch. C. 1894. I. 429.—K. J. IV. 253.
III. IX. Über Mikroorganismen des Tabakes nach der Ernte.—Vers.-Stat. 1895. XXXXVI. 163.—C. f. B. 2. Abt. 1896. II. 34.
IV. Die Infektionskrankheiten des Weines.—C. f. B. 2. Abt. 1896. II. 213.
V. Trockene und nasse Fäule des Tabaks. Der Dachbrand.—Z. f. Pflanzenkrankheiten. 1893. III. 82.—C. f. B. 1894. XVI. 315.
VI. Die Beziehungen der Mikroorganismen zum Tabakbau und zur Tabakfabrikation.—C. f. B. 2. Abt. 1896. II. 514.
VII. Phytopathologische Notizen.—Z. für Pflanzenkrankheiten. 1895. V. 136.
VIII. Studien über die Konservierung und Zusammensetzung des Hopfens.—W. f. Br. 1896. XIII. 802, 946.—Ch. C. 1896. II. 764.—K. J. VII. 61.
IX. Beiträge zur Kenntnis der Obstfäulnis.—C. f. B. 2. Abt. 1898. IV. 514. Also gives prior literature.—Ch. C. 1898. II. 1027.—K. J. IX. 296.
X. Der Bakteriengehalt des ausländischen Getreides. — Wochenblatt d. landw. Vereins im Grossherz. Baden. 1897. No. 24. S. 381.
XI. Der Einfluss der Düngung auf das Faulen des Tabaks.—Vers.-Stat. 1899. L. 223.
XII. Untersuchungen über den Wurzelschimmel der Reben.—C. f. B. 2. Abt. 1897. III. 584.
XIII. Weinlaube. 1903. 412.
XIV. C. f. B. 1892. XI. 110.
XV. W. f. Br. 1895. XII. 802.
XVI. Jahresber. pro 1903 der Landw. Versuchsanstalt Augustenburg. 1904. 43.
XVII. Bot. Ztg. 1901. 2. Abt. LIX. 4.

BEHRING, E.,
I. Über Desinfektion, Desinfektionsmittel und Desinfektionsmethoden.—Z. f. Hygiene. 1891. IX. 395.—Ch. C. 1891. I. 323.—C. f. B. 1891. X. 636.

BEIJERINCK, M. W.,
See BEYERINCK.

BEISSENHERTZ,
I. Berliner Jahresber. 1818. 158.

BELLAMY,
See LECHARTIER et BELLAMY.

BĚLOHOUBEK, Anton,
I. Das Shoyn.—Z. g. Br. 1889. XII. 433.
II. Studien über Presshefe. Prag. 1876.

BENDIX, Ernst,
I. Über die Gärung schwer vergärbarer Zückerarten.—Z. für diätet. u. physikal. Therapie. 1900. III. 587.—C. f. B. 2. Abt. 1900. VI. 503.—Ch. C. 1900. I. 1136.—K. J. XI. 118.

BENDIXEN, N.,
I. Improvements in propagating apparatus for developing pure culture of yeast and bacteria.—Engl. Patent 14,106 of 9. 6. 1897.—K. J. IX. 100.

BENECKE, F.,
I. Über die Ursachen der Veränderungen, welche sich während des Reifungsprozesses im Emmenthaler Käse vollziehen.—C. f. B. 1887. I. 521.
II. Über die Mykorrhiza.—C. f. B. 1888. IV. 753.

BENECKE, F., und SCHULZE, E.,
I. Untersuchungen über den Emmenthaler Käse und über einige andere Schweizerische Käsesorten. — Landw. Jahrbücher. 1887. XVI. 318.—C. f. B. 1887. I. 521.

BENECKE, Wilhelm,
I. Ein Beitrag zur mineralischen Nahrung der Pflanzen. — Ber. d. D. Bot. Ges. 1894. XII. 105.—C. f. B. 2. Abt. 1896. II. 157.—Ch. C. 1895. I. 792.—K. J. V. 70.
II. Die zur Ernährung der Schimmelpilze notwendigen Metalle. — Pringsheim's Jahrb. f. wissensch. Botanik. 1895. XXVIII. 487.—Ch. C. 1896. I. 266.—K. J. VI. 67.
III. Die Bedeutung des Kaliums und des Magnesiums für Entwicklung und Wachstum des Aspergillus niger v. Th. sowie einiger anderer Pilzformen.—Bot. Ztg. 1896. LIV. 1. Abt. 97.—C. f. B. 2. Abt. 1897. III. 675.—K. J. VII. 46.

Bérard, Jacques Étienne,
I. Sur la maturation des fruits.—Annales de Chimie et de Physique. 1821. XVI. 152.

Bergh, Axel,
See Jörgensen und Bergh.

Bergmann, E.,
I. Das putride Gift und die putride Intoxikation. Dorpat. 1866.

Bergmann, E., und Schmiedeberg, O.,
I. Über das schwefelsaure Sepsin.—Centralblatt f. d. med. Wissenschaften. 1868. p. 394.

Bergmann, Emil,
I. Untersuchungen über das Vorkommen der Ameisensäure und Essigsäure in den Pflanzen und über die physiologische Bedeutung derselben im Stoffwechsel.—Bot. Ztg. 1882. XL. 731. Gives prior literature.—Z. g. Br. 1884. VII. 86.

Berlese, Amedeo,
I. Prima contribuzione allo studio della morfologia e biologia di Cladosporium e Dematium.—Revista di Patologia vegetale. Firenze. 1895. III. 2.—Zeitschr. f. Pflanzenkrankheiten. 1896. VI. 104.
II. Verhalten der Saccharomyceten an den Weinstöcken. — I.–III. Abhandlg. — Rivista di Patologia vegetale. 1897. VII.—C. f. B. 2. Abt. 1897. III. 592. —K. J. VIII. 116.
III. Bull. Soc. Mycologique de France. 1895. XI. 34.
IV. Rivista di Patologia vegetale. 1896–1897. V. Nos. 5–12. 1897. VI. Nos. 6–10.

Berlese et Sostegni,
I. Recherches sur l'action des sels de cuivre sur la végétation de la vigne et sur le sol.—La Revue internationale de viticulture et œnologie.—C. f. B. 2. Abt. 1895. I. 770.

Bernard, Claude,
I. Sur le mécanisme de la formation du sucre dans le foie.—C. R. 1855. XLI. 461; 1857. XLIV. 578.
II. Leçons sur les phénomènes de la vie. 1878.

Bernheim, H.,
I. Taschenbuch für den bakteriologischen Praktikanten. 2. Ed. 1891. Würzburg. Stuber. 1*s*.
II. Die parasitären Bakterien der Cerealien.—Münchn. mediz. Wochenschrift. 1888. p. 743.—C. f. B. 1889. V. 126. —Z. g. Br. 1888. XI. 502.

Bernheimer, O.,
I. Allgem. Weinztg. 1895.—Ch. C. 1895. II. 650.

Bernstein, Alexander,
I. Die Herstellung eines neuen Getränkes aus Milch.—Milch Zeitung. 1895. XXIV. 85 u. 942.—Ch. C. 1895. I. 707; 1896. I. 317.—K. J. VI. 224.
II. Umwandlung des Caseins der Milch in Albumosen und Peptone mittelst einer Bakterie. German Patent, No. 80,451. —K. J. VI. 224.

Bert, Paul,
I. Influence de l'air comprimé sur les fermentations.—C. R. 1875. LXXX. 1579.

Berthelot, Marcelin,
I. Fixation directe de l'azote atmosphérique libre par certains terrains argileux. —C. R. 1885. CI. 775; 1886. CII. 951; 1887. CIV. 205, 625; 1889. CVIII. 700; CIX. 277; 1892. CXV. 569.—Ch. C. 1885. 904; 1886. 460; 1887. 304, 418; 1889. I. 725, II. 563.
II. Recherches nouvelles sur les microorganismes fixateurs de l'azote.—C. R. 1893. CXVI. 842.—K. J. IV. 230.
III. Recherches sur la fermentation.—C. R. 1856. XLIII. 238.
IV. Faits pour servir à l'histoire du raffinose.—C. R. 1889. CIX. 548.—Z. g. Br. 1889. XII. 481.—Ch. C. 1889. II. 869.
V. Chimie organique, fondée sur la synthèse. Paris. 1860. Tome II. p. 621.
VI. C. R. 1857. XLIV. 702.
VII. Ann. de Chimie et de Physique. 1865. 4ème série. VI. 329; 399.
VIII. C. R. 1863. LVII. 797.
IX. C. R. 1860. L. 980.
X. *Ibia.* 1855. XLI. 392.

Berthelot, M., et André, G.,
I. Sur l'odeur propre de la terre.—C. R. 1891. CXII. 598.—Ch. C. 1891. I. 891.

Bertrand, Gabriel,
I. Préparation biochimique du sorbose.—C. R. 1896. CXXII. 900.—Ch. C. 1896. I. 1201.—K. J. VII. 228.
II. Sur le latex de l'arbre à laque.—C. R. 1894. CXVIII. 1215; CXX. 266.—Ch. C. 1894. II. 99; 1895. I. 606.
III. Sur les rapports qui existent entre la constitution chimique des composés organiques et leur oxydabilité sous l'influence de la laccase.—C. R. 1896. CXXII. 1132.—Ch. C. 1896. II. 93.—K. J. VII. 244.
IV. Sur une nouvelle oxydase, ou ferment soluble oxydant d'origine végétale.—C. R. 1896. CXXII. 1215.—Ch. C. 1896. II. 108.—K. J. VII. 245.
V. Sur la recherche et la présence de la laccase dans les végétaux.—C. R. 1895. CXXI. 166.—Ch. C. 1895. II. 496; 1896. I. 252.
VI. Sur l'intervention du manganèse dans les oxydations provoquées par la laccase. —C. R. 1897. CXXIV. 1032, 1355. —Ch. C. 1897. II. 47, 177.
VII. C. R. 1898. CXXVI. 842, 984.

Bertrand, G., et Mallèvre, A.,
I. La Pectase.—C. R. 1894. CXIX. 1012; 1895. CXX. 110; CXXI. 726.—Ch. C. 1895. I. 276, 482; 1896. I. 123.

Berzelius, Jöns Jakob,
I. Lehrbuch der Chemie. 1840.
Besana, Carlo,
I. Über das Grünwerden des Lodisaner Käses.—Jahrbuch der milchw. Versuchsstation zu Lodi. 1888. — Milch-Ztg. 1888. XVII. 364.
Beu, Hans,
I. Über den Einfluss des Räucherns auf die Fäulniserreger bei der Konservierung von Fleischwaren. — C. f. B. 1890. VIII. 513.—K. J. I. 43.
Beumer,
I. Die Bakteriologie des Bodens. — Deutsche med. Wochenschrift. 1886. No. 27.
Bevan, E. J.,
See Cross and Bevan ; Cross, Bevan and Smith.
Beyerinck, M. W.,
I. Über Atmungsfiguren beweglicher Bakterien.—C. f. B. 1893. XIV. 827. — K. J. IV. 75.
II. Über Spirillum desulfuricans als Ursache von Sulfatreduktion.—C. f. B. 2. Abt. 1895. I. 1.—Ch. C. 1895. II. 169.
III. Über Thermotaxis bei Bacterium Zopfii.—C. f. B. 1894. XV. 799.—K. J. V. 88.
IV. Die filtrierende Wirkung der Chamberland'schen Bougies.—Wissenschaftl. Nachrichten d. Nieuwe Rotterd. Courier. 1890.—K. J. I. 17.
V. Verfahren zum Nachweise der Säureabsonderung bei Mikrobien.—C. f. B. 1891. IX. 781.—Ch. C. 1892. I. 635. —K. J. II. 11.
VI. Kulturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen.—Bot. Ztg. 1890. XXXXVIII. 725.—C. f. B. 1890. VIII. 460; 1893. XIII. 368.—K. J. IV. 83.
VII. Kulturversuche mit Amöben auf festem Substrate.—C. f. B. 1. Abt. 1896. XIX. 257.
VIII. L'auxanographie ou la méthode de l'hydrodiffusion dans la gélatine appliquée aux recherches microbiologiques. — Archives Néerlandaises. 1889. XXIII. 367.—C. f. B. 1890. VII. 347.
IX. Qualitative und quantitative mikrobiochemische Analyse.—C. f. B. 1891. X. 723.—Ch. C. 1892. I. 142.—K. J. II. 11.
X. Die Lebensgeschichte einer Pigmentbakterie.—Bot. Ztg. 1891. XXXXIX. 705.—C. f. B. 1892. XII. 862.—K. J. II. 78.
XI. Over lichtvoedsel en plastisch voedsel van Lichtbakterien.—Verslagen en Mededeelingen der Koninklijke Akademie van Wetenschappen. Afdeeling Natuurkunde. 2de Reeks. Deel VII. 239.—C. f. B. 1890. VIII. 616.—K. J. I. 180.
XII. Über die Butylalkoholgärung und das Butylferment.—Verhandlungen der K. Akademie van Wetenschappen te Amsterdam. Tweede Sectie, Deel I, No. 10.—Archives Néerlandaises des sciences exactes et naturelles. 1895. XXIX.—C. f. B. 1894. XV. 171. — Ch. C. 1894. I. 963.—K. J. IV. 258.
XIII. Über Nachweis und Verbreitung der Glukase, das Enzym der Maltose.—C. f. B. 2. Abt. 1895. I. 221.—K. J. VI. 320.
XIV. Die Bakterien der Papilionaceen-Knöllchen.—Bot. Ztg. 1888. XXXXVI. 725. With plate.—C. f. B. 1889. V. 804.
XV. Künstliche Infektion von Vicia Faba mit Bacillus radicicola—Bot. Ztg. 1890. XXXXVIII. 837.—K. J. I. 128.
XVI. Über die Natur der Fäden der Papilionaceenknöllchen.—C. f. B. 1894. XV. 728.—K. J. V. 248.
XVII. Over ophooping van atmopherisches Stickstof in culturen van Bacillus radicicola.—Verslagen en Mededeelingen der Koninkl. Akademie van Wetenschappen te Amsterdam. Afdeel: Natuurkunde. 1891. 3de Reeks. VIII. 460.—C. f. B. 1892. XII. 687.—K. J. III. 203.
XVIII. Schizosaccharomyces octosporus, eine achtsporige Alkoholhefe.—C. f. B. 1894. XVI. 49.—Ch. C. 1894. II. 614.—K. J. V. 38.
XIX. Sur le Kéfir.—Archives Néerlandaises etc. 1889. XXIII. 428.—Ch. C. 1893. I. 619.—K. J. III. 182.
XX. Die Lactase, ein neues Enzym.—C. f. B. 1. Abt. 1889. VI. 44.—Ch. C. 1889. II. 461.
XXI. Zur Ernährungsphysiologie des Kahmpilzes.—C. f. B. 1. Abt. 1892. XI. 68.—Ch. C. 1892. I. 445.—K. J. III. 108.
XXII. Weitere Beobachtungen über die Octosporushefe.—C. f. B. 2. Abt. 1897. III. 449.—Ch. C. 1897. II. 868.—K. J. VIII. 98.
XXIII. Notiz über die Cholerarot-Reaktion.—C. f. B. 1. Abt. 1892. XII. 715. —Ch. C. 1893. I. 106.—K. J. III. 64.
XXIV. C. f. B. 1898. IV. 657.
XXV. Bot. Centralbl. 1892. LI. 384.
XXVI. C. f. B. 2. Abt. 1897. III. 454.
XXVII. *Ibid.* 1900. VI. 11.
XXVIII. *Ibid.* 1894. XVI. 51.
XXIX. Verhand. der K. Akad. v. Wetenschappen te Amsterdam. Tweede Sectie, Deel I. 1893; Deel II. 1894. No. 40. Recueil des travaux chim. des Pays-Bas. 1893. XII. 141.
XXX. Bull. de l'Assoc. Belge des Chimistes. 1890. XVI. 178.—Archives Néerlandaises. 1890. XXIII. 428.
XXXI. C. f. B. 2. Abt. 1897. III. 449, 521.
XXXII. *Ibid.* 1900. VI. 194.

Biedermann, W.,
I. Beiträge zur vergleichenden Physiologie der Verdauung. I.—Pflüger's Archiv. 1898. LXXII. 105.—Ch. C. 1898. II. 370.

Biel, J.,
I. Studien über die Eiweissstoffe des Kumys und des Kefir. — Pharmac. Zeitschrift f. Russland. 1886. XXV. 161.—Milchzeitung. 1886. XV. 128.
II. Über Kefir, seine Eigenschaften und Bereitung.—*Ibid.* p. 267.—Ch. C. 1886. p. 845.

Biel, W.,
I. Über einen schwarzes Pigment bildenden Kartoffelbacillus.—Inaug.-Diss. Kiel. 1896.—C. f. B. 2. Abt. II. 137.—K. J. VII. 27.

Bienstock, Berthold,
I. Über die Bakterien der Fäces.—Zeitschrift für klinische Medizin. 1883. VIII. 1.—Ch. C. 1883. p. 809; 1884. p. 646.

Bierbrouwery d'Oranjeboom,
I. Beziehungen der Hefengabe zum Hefenwachstum und zum Attenuationsverlauf während der Biergärung.—Z. g. Br. 1897. XX. 423.—K. J. VIII. 124.

Biernacki, E.,
I. Über die Eigenschaft der Antiseptica, die Alkohol-Gärung zu beschleunigen und über gewisse Abhängigkeit ihrer Kraft von der chemischen Baustruktur, der Fermentmenge und der Vereinigung mit einander.—Pflüger's Archiv f. d. ges. Physiologie. 1891. XXXIX. 112.—Reproduced in Z. g. Br. 1891. XIV. 248.—C. f. B 1891. X. 296.—Ch. C. 1891. II. 31.—K. J. II. 69.

Biffen, R. H.,
I. A Fat-destroying Fungus.—Annals of Botany. 1899. XIII. 363.

Biginelli, P.,
I. Composizione e costituzione chimica del gas arsenicale delle tappezzerie.—Atti della R. Accad. dei Lincei. Roma. 1900. [5]. IX. 210, 242.—Ch. C. 1900. II. 1067, 1100.

Billroth, Theodor,
I. Untersuchungen über die Vegetationsformen von Coccobacteria septica, etc. Berlin 1874. G. Reimer.

Biot et Persoz,
I. Ann. de Chim. et de Phys. 1833. LIII. 83.

Biourge, Ph.,
I. Recherches sur la fermentation alcoolique.—La Cellule. 1895. XI. 95.—Ch. C. 1896. II. 105.—K. J. VI. 149.

Birch-Hirschfeld,
I. Über die Züchtung von Typhusbacillen in gefärbten Nährlösungen. — A. f. Hygiene. 1888. VII. 341.—C. f. B. 1888. III. 447 u. 569.

Bischoff, C. A.,
I. Weitere Beiträge zur Kenntnis der substituierten Bernsteinsäuren.—Ber. d. D. Chem. Ges. 1891. XXIV. 1064.—Ch. C. 1891. I. 963.

Bissinger,
I. Über Bestandteile der Pilze Lactarius piperatus und Elaphomyces granulatus.—Archiv. der Pharmacie. 1883. p. 321.

Bittó, Béla von,
See Liebermann und Bitto.

Bizzozero, Giulio,
I. Sui microfiti dell' epidermide umana.—Atti Acc. Torino. 1894.

Blachstein,
I. Contribution à la biologie du bacille typhique.—Archives de sciences biol. publ. par l'Institut imp. de méd. expér. à St. Pétersbourg. 1892. I. No. 1 et 2.—K. J. III. 80.

Bleisch, C., u. Schweitzer, R.,
I. Beiträge zur Kenntnis der Vorgänge beim Lüften der Würze.—Z. g. Br. 1899. XXII. 515.—Ch. C. 1900. II. 697.

Bleisch, M.,
I. Über bittere Milch und die Sterilisierung der Milch durch Erhitzen unter Luftabschluss.—Z. f. Hygiene. 1893. XIII. 81.—K. J. IV. 182.

Blondeau, Ch.,
I. Etude chimique du fromage de Roquefort.—Annales de chimie et de physique. 4ème série. 1864. I. 208.—Ch. C. 1864. p. 333.
II. Sur la formation de l'humus et du nitre.—C. R. 1863. LVII. 414.

Blondlot,
I. Sur la fermentation alcoolique du sucre de lait.—C. R. 1872. LXXIV. 534.

Blumenthal,
I. Virchow's Archiv. 1894. CXXXVII. 539.

Blumentritt,
I. Ber. d. D. Bot. Ges. 1901. XIX. 442.
II. *Ibid.* 1905. XXIII. 419.

Blunt,
See Downes and Blunt.

Bochicchio, Nicola,
I. Über einen Milchzucker vergärenden und Käseblähungen hervorrufenden neuen Hefepilz.—C. f. B. 1. Abt. 1894. XV. 546.—Le stazioni sperim. agrar. ital. 1894. XXVI. 339, 568.—Ch. C. 1894. II. 214.—K. J. V. 244.

Bodin,
I. Les parasites de l'Homme. Paris. 1902.

Boersch, C.,
I. Beitrag zur Kenntnis der Bakterien des Weines und zur Kenntnis der Hefen. Dissert. Erlangen. 1893.—K. J. IV. 127.

Böhmer,
I. Die Heubereitungsarten.—Deutsche landw. Presse. 1890. XVII. 103.

Boidin, A., and Rolants, E.,
I. The Utilisation of the Chinese Yeast, Amylomyces Rouxii, in European Industries.—La Bière. 1897. V. 33.—K. J. VIII. 131.
See also Collette and Boidin.

Bókay, A.,
I. Über die Verdaulichkeit des Nucleins und Lecithins.—Z. f. physiolog. Chemie. 1877. I. 157.

Bokorny, Theodor,
I. Über den Einfluss des Calciums und Magnesiums auf die Ausbildung der Zellorgane.—Botan. Centralblatt. 1895. LXII. 1.
II. Chemisch-physiologisches über die Hefe.—Pharmaceut. Centralhalle. 1900. XXXXI. 737.—Ch. C. 1901. I. 56.
III. Über die Kohlenstoffernährung der Sprosshefe. — Dingler's polytechn. Journal. 1897. CCCIII. 115.—C. f. B. 2. Abt. 1897. III. 372.—Ch. C. 1897. I. 553.—K. J. VIII. 86.
IV. Chem. Zeitung. 1901. XXV. 502; 1902. XXVI. 704; 1903. XXVII. 502.

Bolas, Th.,
I. Alkoholgehalt des Brotes.—Chemical News. 1873. XXVII. 271.—Ch. C. 1873. p. 676.

Bolley, H. L., and Hall, C. M.,
I. Cheese curd inflation. Its relation to the bacterial flora of fore milk.—C. f. B. 2. Abt. 1895. I. 788.

Boltshausen, H.,
See Kirchner und Boltshausen.

Bondzynski, Stephan,
I. Zur Kenntnis der chemischen Natur einiger Käsearten.—Landw. Jahrbuch der Schweiz. 1894. VIII. 189.

Bonhoff,
I. Die Einwirkung höherer Wärme grade auf Tuberkelbacillen-Reinkulturen.—Hygien. Rundschau. 1892. II. 1009.—C. f. B. 1893. XIII. 294.
II. Untersuchungen über Giftbildung verschiedener Vibrionen in Hühnereiern.—A. f. Hygiene. 1895. XXII. 351.

Bonnet, Charles,
I. Considérations sur les corps organisés. 1762. Amsterdam et Paris.

Bonnier et Mangin,
I. Recherches sur la respiration et transpiration des champignons.—Annales des sciences naturelles. Botanique. 6ème série. 1884. XVII. 210.
II. Recherches sur la respiration des tissues sans chlorophylle. *Ibid.* 1884. XVIII. 293.

Bonorden,
I. Handbuch d. allg. Mykologie. Stuttgart. 1851.

Boorsma, W. G.,
I. Ang-Khak. — Geneesk Tijds. 1896. XXXV.—Nederlandsch Tijdschrift voor Pharmacie, etc. 1896. VIII. 126.—Ch. C. 1896. I. 1131.

Bordas,
I. Sur une maladie nouvelle du vin en Algérie.—C. R. 1888. CVI. 85.
II. C. R. 1904. CXXXVIII. 928.

Bordoni-Uffreduzzi, Guido,
I. Ein Fall von fuchsinähnlicher Bakterienfärbung des Fleisches—Hygien. Rundschau. 1894. IV. 12.—C. f. B. 1894. XV. 666.—Ch. C. 1894. I. 914.—K. J. V. 87.
II. Die biologische Untersuchung des Eises in seiner Beziehung zur öffentlichen Gesundheitspflege.—C. f. B. 1887. II. 489.

Borgeaud.
See Hess und Borgeaud.

Borgman,
I. Zeitg. f. analyst. Chem. 1886. XXV. 532.

Borntträger,
I. Zeitg. f. angew. Chem. 1894. 13.
II. Deutscher Chemiker-Ztg. 1893. VII. 377.

Borschtschoff, I.,
I. Die gallertigen Substanzen der Rübensäfte und Sirupe.—Annalen der Technischen Gesellschaft zu Kiew.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1876. p. 738.

Botkin, S.,
I. Über einen Bacillus butyricus.—Z. f. Hygiene. 1892. XI. 421.—C. f. B. 1892. XI. 628.—K. J. III. 233.

Bouchardat,
I. C. R. 1874. LXXVIII. 1145.

Bouffard, A.,
I. Sur le cassage des vins.—C. R. 1894. CXVIII. 827.—K. J. V. 194.
II. C. R. 1895. CXXI. 357.

Boullanger, E.,
I. Action des levures de bière sur le lait.—Ann. Inst. Past. 1897. XI. 720.—Ch. C. 1897. II. 1011.—K. J. VIII. 155.
II. Contribution à l'étude de quelques levures de bière.—Ann. Inst. Past. 1896. X. 598.—C. f. B. 2. Abt. 1897. III. 23.—K. J. VII. 95.

Bouquet, Robert,
I. Nouvelle hypothèse sur l'absorption de l'azote par les végétaux.—Journal de l'agriculture pratique. 1888. I. 710.—Biedermann's Centralbl. 1891. p. 424.

Bourquelot, Émile,
I. Les Ferments solubles.—Paris. 1896. Société d'éditions scientifiques.—K. J. VII. 247.
II. Sur l'hydrolyse de la raffinose (mélitose) par les ferments solubles.—Journal de Pharmacie et de Chimie. 6e série. 1896. III. 390.—Ch. C. 1896. I. 1169.—K. J. VII. 238.

III. Présence et rôle de l'émulsine dans quelques champignons parasites des arbres ou vivant sur le bois.—Comptes rendus de la société de biologie. 1893, p. 804.—K. J. IV. 284.
IV. Présence d'un ferment analogue à l'émulsine dans les champignons parasites des arbres ou vivant sur le bois. C. R. 1893. CXVII. 383.—Ch. C. 1893. II. 825.—K. J. IV. 284.
V. C. R. 1893. CXVI. 826.
VI. Bull. Soc. Mycol. de France. 1893. IX. 230.
VII. Comptes rendus Soc. de Biologie. 1896. XLVIII. 205.
VIII. C. R. 1902. CXXXV. 349.—Journ. de Pharm. et de Chimie. 1903. XVI. 578.
IX. Comptes rendus Soc. de Biologie. 1903. LV. 386.
X. *Ibid.* 1896. XLVIII. 896.
XI. C. R. Soc. de Biologie. 1893. XLV. 653, 804.
XII. C. R. Soc. de Biologie. XLV. 425.
XIII. Comptes rendus Soc. de Biologie. 1893. 653.—C. R. 1893. CXVI. 1143; CXVII. 826.
XIV. C. R. 1903. CXXXVI. 762.
XV. C. R. 1898. CXXVI. 1045; 1901. CXXXIII. 690; 1902. CXXXV. 399.

Bourquelot, E., et Bertrand, G.,
I. La laccase dans les Champignons.—C. R. 1895. CXXI. 783.—Ch. C. 1896. I. 124.
II. Über die Färbung der Gewebe und des Saftes gewisser Pilze an der Luft.—Journal de Pharmacie et Chimie. 1896. III. 177.—Ch. C. 1896. I. 756.—K. J. VII. 244.

Bourquelot et Graziani,
I. Bull. Soc. Mycol. de France. 1896. XII. 27.

Bourquelot, E., et Hérissey, H.,
I. Recherche et présence d'un ferment soluble protéohydrolytique dans les Champignons.—C. R. 1898. CXXVII. 666.—Journal de Pharmacie et de Chimie. 1898. sér. VI. t. VIII. p. 448.—C. f. B. 2. Abt. 1899. V. 159.—Ch. C. 1898. II. 1274.
II. Journ. de Pharm. et de Chimie. 1902. XVI. 417.
III. *Ibid.* 1897. IX. 385.
IV. C. R. 1895. CXXI. 696.
V. *Ibid.* 1901. CXXXII. 571.
VI. C. R. 1899. CXXIX. 228.

Boussingault,
I. C. R. 1880. XCI. 373.
II. Ann. de Chim. et de Phys. 1881. [5]. XXII. 118.

Boutron-Chalard, Antoine François. *See* Frémy et Boutron-Chalard.

Boutroux, Léon,
I. Sur une fermentation nouvelle du glucose.—C. R. 1878. LXXXVI. 605; 1880. LXXXXI. 236.—Ch. C. 1880. p. 600.
II. Contribution à l'étude de la fermentation panaire.—C. R. 1883. LXXXXVII. 116.—Ch. C. 1883. p. 614.
III. Sur la fermentation panaire.—C. R. 1891. CXIII. 203.—C. f. B. 1892. XII. 153.—Ch. C. 1891. II. 549.—K. J. II. 227.
IV. Sur les causes qui produisent la couleur du pain bis.—C. R. 1895. CXX. 934.
V. Sur la conservation des ferments alcooliques.—Annales des sciences naturelles. Botanique. 6e série. 1884. XVII. 144.
VI. Sur la dissémination naturelle des levures de vin.—C. R. 1898. CXXVII. 1033.—C. f. B. 2. Abt. 1899. V. 311.
VII. Le pain et la panification. Paris. 1897. Baillière et fils. 4*s.* 2*d.*
VIII. Bull. de la Soc. Linn. de Normandie. 1883. VII.—Ann. d. Sc. Nat. Bot. 1884. XVII. 196.
IX. Bull. de la Soc. Linn. de Normandie. 1881. VI. 1883. VII.
X. A. d. Sci. Nat. Bot. 1884. XVII. 144.

Bovet, V.,
I. Ann. de Microgr. 1891. III. 353.

Bowhill, Thomas,
I. Zur bakteriolog. Technik.—C. f. B. 2. Abt. 1899. V. 287.

Boyce, R., and Evans, E.,
I. Upon the action of gravity on Bacterium Zopfii.—Proceedings of the Royal Society of London. 1893. LIV. 300.—C. f. B. 1894. XV. 568.—K. J. IV. 123.

Braconnot, Henri,
I. Sur la Jusée et l'Écorce de chêne.—Annales de chimie et de physique. 1832. L. 376.
II. De la Fongine, ou Analyse des Champignons.—Journal de Physique, de chimie, etc. 1811. LXXIII. 130.
III. Recherches analytiques sur la nature des champignons.—Annales de chimie. 1811. LXXIX. 265; LXXX. 272.

Bräutigam, Walter,
I. Über das Gelatinieren des Infusum foliorum Digitalis.—Pharm. Centralhalle. 1891. XXXII. 349, 427.—Ch. C. 891. II. 489, 549.—K. J. II. 222.
II. Unters. üb. d. Mikroorganismen in Schlämpe u. Bierträben. Dissert. Leipzig. 1886.

Brand, Josef,
I. Krickenbier.—From "La Gazette du Brasseurs." 1897. XI. 61, noticed in Z. g. Br. 1897. XX. 543.—K. J. VIII. 140.
II. Über das Vorkommen von Furfurol im Malze, in der Würze und im Biere.—Z. g. Br. 1898. XXI. 255.—Ch. C. 1898. II. 146.—K. J. IX. 161.
III. Z. g. Br. 1893. XVI. 303; 1894. XVII. 131.

BRANDES, P., und STOEHR, C.,
I. Über die Bildung von Pyrazin und Homologen aus Traubenzucker und Ammoniak.—Journal f. prakt. Chemie. 1896. CLXII. 481.—Ch. C. 1897. I. 381.
II. Z. f. prakt. Chem. 1896. CLXI. 501.

BRASSIER,
I. Sur les modifications que le fromage subit en vieillissant.—Ann. d. chim. et phys. 4ème s. 1865. V. 270.

BRAULT, A., et LOEPER, M.,
I. Journ. de Physiol. et de Pathol. générale. 1904. VI. 720.

BRAUN, Richard,
I. Nachweis des Glycogenes in Hefenzellen.—Z. g. Br. 1901. XXIV. 397. —C. f. B. 2. Abt. 1902. VIII. 27.—Ch. C. 1901. II. 446.
II. Z. g. Br. 1902. XXV. 409.

BRÉAL, E.,
I. De la présence, dans la paille, d'un ferment aérobie, réducteur des nitrates. —C. R. 1892. CXIV. 681.—C. f. B. 1892. XII. 300.—K. J. III. 227.

BREFELD, Oskar,
I. Botanische Untersuchungen über Schimmelpilze. I. Heft. 1872: Mucor Mucedo, etc. Zygomyceten.
II. *Ibid.* II. 1874: Die Entwicklungsgeschichte von Penicillium.
III. *Ibid.* III. 1877: Basidiomyceten. I.
IV. *Ibid.* IV. 1881: Kulturmethoden zur Untersuchung der Pilze. Bacillus subtilis, etc.
V. *Ibid.* V. 1883: Die Brandpilze. I. Darunter: Der morphologische Wert der Hefen.
VI. *Ibid.* VI. 1884: Myxomyceten. I. Entomophthoreen. II.
VII. *Ibid.* VII. 1888: Basidiomyceten. II.
VIII. *Ibid.* VIII. 1889: Basidiomyceten. III. Das natürliche System der Pilze.
IX. *Ibid.* IX. 1891: Die Hemiasci und die Ascomyceten.—C. f. B. 1. Abt. 1892. XI. 291.—K. J. III. 41.
X. *Ibid.* X. 1891: Ascomyceten. II.
XI. *Ibid.* XI. 1895: Die Brandpilze. II.
XII. *Ibid.* XII. 1895: Hemibasidii. Brandpilze. III.
XIII. Untersuchungen über Alkoholgärung.—Landw. Jahrbücher. 1874. III. 65.—Ber. d. D. Chem. Ges. 1874. VII. 281.—Botan. Jahresber. pro 1874. 341, 342.
XIV. *Ibid.* II.—Landw. Jahrbücher. 1875. IV. 405.—Botan. Jahresbericht pro 1875. p. 198.
XV. *Ibid.* III.—Landw. Jahrbücher. 1876. V. 281.—Botan. Jahresbericht pro 1876. p. 143.
XVI. Mucor racemosus und Hefe.—Flora. 1873. LVI. 385.
XVII. Landw. Jahrbücher. 1875. IV. 414; 1876. V. 332.
XVIII. Landw. Jahrbücher. 1876. LIX. 310.

BREMER,
I. Dissert. Münster. 1902.

BREVANS, I. de,
I. Les Conserves alimentaires. Paris. 1896. Baillière et fils.

BRIANT, Lawrence,
I. The Influence of Aeration on Fermentation. — Journal of the Federated Institutes of Brewing. 1895. I. 472.—Z. g. Br. 1896. XIX. 100.—K. J. VI. 136.
II. The Influence of Nitrates in Brewing. —Journ. Fed. Institutes of Brewing. 1899. V. 372—K. J. X. 134.

BRIEGER, Ludwig.
I. Spaltungsprodukte der Bakterien.—Z. f. physiolog. Chemie. 1884. VIII. 306. 1885. IX. 1.—Ch. C. 1884. p. 456. 1885. p. 60.
II. Über die flüchtigen Bestandteile der menschlichen Exkremente.—Ber. d. D. Chem. Ges. 1877 X. 1027.—Journal f. prakt. Chemie. 1878. XVII. 124.—Ch. C. 1887. p. 469.
III. Über Ptomaïne. Berlin. 1885.—Ch. C. 1885. p. 362.
IV. Zur Kenntnis der Fäulnisalkaloide.—Z. f. physiolog. Chemie. 1883. VII. 274. —Ch. C. 1883. p. 539.
V. Die Quelle des Trimethylamins im Mutterkorn.—Z. f. physiolog. Chemie. 1887. XI. 184.
See also BAUMANN und BRIEGER.

BRIZI, Ugo,
I. Über die Fäulnis der Rebentriebe, durch Botrytis cinerea verursacht.—C. f. B. 2. Abt. 1897. III. 141.—Ch. C. 1897. I. 996.

BROCHET, A.,
I. Sur la production de l'aldehyde formique gazeux pur.—C. R. 1896. CXXII. 201.—Ch. C. 1896. I. 539.—K. J. VII. 15.
See also CAMBIER et BROCHET.

BRODMEIER, A.,
I. Über die Beziehung des Proteus vulgaris Hsr. zur ammoniakalischen Harnstoffzersetzung.—Dissert. Erlangen. 1896. —C. f. B. 1. Abt. 1895. XVIII. 380.—Ch. C. 1896. I. 316.

BROWN, Adrian J.,
I. On an Acetic Ferment which forms Cellulose.—Journal of the Chemical Society. 1886. XXXXIX. 432.—Ch. C. 1886. p. 699; 1887. p. 804.
II. Note on Bacillus subtilis.—Journal of the Federated Institutes of Brewing. 1895. I. 423.—Z. g. Br. 1895. XVIII. 339.—W. f. Br. 1895.—XII. 657.—K. J. VI. 206.

III. The Chemical Action of Pure Cultivations of Bacterium aceti.—Journal of the Chemical Society. 1886. XXXXIX. 172; 1887. LI. 638.—Ch. C. 1886. p. 200; 1887. p. 803.
IV. Influence of Oxygen and Concentration on Alcoholic Fermentation.—Journal of the Chem. Society. Transactions. 1892. LXI. 369.—C. f. B. 1. Abt. 1892. XII. 148.—Ch. C. 1892. I. 560.—K. J. III. 101.
V. Über das Fermentationsvermögen.—C. f. B. 2. Abt. 1897. III. 33.—Ch. C. 1897. I. 871.—K. J. VIII. 91.
VI. The Specific Character of the Fermentative Functions of Yeast-cells.—Brewing Trade Review. 1895. IX. No. 104.—K. J. VI. 57.
VII. Versuche über die numerische Vermehrung der Hefezellen.—Transactions of the Laboratory Club. 1890. III. 64.—Ch. C. 1890. I. 803.—W. f. Br. 1890. VII. 355.—K. J. I. 58.
VIII. Journ. Federated Inst. Brewing. 1901. VII. 93.

Brown, Horace T., and Morris, G. H.,
I. On Certain Functions of Hops used in the Dry-hopping of Beers.—Transactions of the Institute of Brewing. 1893. VI. No. 4.—K. J. IV. 137.
II. Über eine neue Methode der Bier-Analyse.—Transactions of the Laboratory Club. 1890.—K. J. I. 68.
III. Chem. Zeitg. 1893. XVII. 655.
IV. Liebig's Ann. 1885. CCXXXI. 134.
V. Journ. Chem. Soc. 1889. 449, 462.

Brown, Robert,
I. Observations on the Organs and Mode of Fecundation in Orchideæ and Asclepiadeæ. London. 1831.

Brücke, Ernst,
I. Die Elementarorganismen. — Sitzungsberichte der K. Akademie der Wissenschaften. Mathem.-naturw. Kl. 1861. XXXIV. Abt. II. p. 381.

Brunchorst, Jörgen,
I. Über die Knöllchen an den Leguminosenwurzeln.—Ber. d. D. Bot. Ges. 1885. III. 241.
II. Über die Wurzelanschwellungen von Alnus und den Elaeagnaceen. Dissert. Tübingen. 1886.—Untersuchungen a. d. Botan. Inst. zu Tübingen.

Brunner, Alfred,
See Morpurgo und Brunner.

Brunstein,
I. Beihefte z. Bot. Centralbl. 1901. X. 1.

Brusilowsky, E.,
I. Die Bedeutung der Mikroorganismen bei der Bildung des Buchtschlammes.—Wratsch 1890. p. 717.—Hygienische Rundschau. 1891. I. 240.—C. f. B. 1891. X. 194.—Ch. C. 1891. I. 672.—K. J. II. 227.

Bryilants, O.,
I. Milchztg. 1895. XXIV. 697.

Buchner, Eduard,
I. Über den Einfluss des Sauerstoffes auf Gärungen.—Z. f. physiolog. Chemie. 1885. IX. 380. — Z. g. Br. 1885. VIII. 268.—Ch. C. 1885. p. 555.
II. Alkoholische Gärung ohne Hefezellen.—Ber. d. D. Chem. Ges. 1897. XXX. 117.—C. f. B. 2. Abt. 1897. III. 251.—Ch. C. 1897. I. 422.
III. *Ibid.* II. Mittlg.—Ber. d. D. Chem. Ges. 1897. XXX. 1110.—C. f. B. 2. Abt. 1897. III. 527.—Ch. C. 1897. II. 46.—Z. g. Br. 1897. XX. 362.
IV. Notiz aus der Gärungschemie.—Ber. d. D. Chem. Ges. 1892. XXV. 1161.—Ch. C. 1892. I. 860.—K. J. III. 241.
V. Zymase aus getöteter Hefe.—Ber. d. D. Chem. Ges. 1900. XXXIII. 3307.—C. f. B. 2. Abt. 1901. VII. 247.—Ch. C. 1901. I. 54.—K. J. XII.
VI. Bemerkungen zur Arbeit von A. Macfadyen, G. H. Morris und S. Rowland: "Über ausgrepresstes Hefezellplasma (Buchner's Zymase)."—Ber. d. D. Chem. Ges. 1900. XXXIII. 3311.—C. f. B. 2. Abt. 1901. VII. 73.—Ch. C. 1901. I. 55.—K. J. XI. 375.
VII. Über die Zymase.—W. f. Br. 1901. XVIII. 197.—C. f. B. 2. Abt. 1901. VII. 845.—Ch. C. 1901. I. 1110.
VIII. Verfahren zur Gewinnung des flüssigen Zellinhaltes von Mikroorganismen in unveränderter Form. German Pat., No. 99,508, Jan. 12, 1897.—Ch. C. 1899. I. 320.—K. J. IX. 314.
IX. Verfahren zur Herstellung abgetöteter Dauerhefe. German Pat., No. 97,240, April 30, 1897.—Ch. C. 1898. II. 591.—K. J. IX. 315.
X. Zymasegärung. München und Berlin. 1903.

Buchner, E., u. Antoni, W.,
I. Z. f. physiol. Chem. 1905. XLIV. 206.
II. Z. f. physiol. Chem. XLVI. 136.

Buchner u. Hahn,
I. Die Zymasegärung. 1903. 210.

Buchner, E., u. Meisenheimer, J.,
I. Zeit. f. physiolog. Chemie. 1903. XL. 167.
II. Ber. d. D. Chem. Ges. 1905. XXXVIII. 620.
III. *Ibid.* 1903. XXXV. 634.
IV. *Ibid.* 1904. XXXVII. 417.

Buchner, E., u. Mitscherlich, S.,
I. Z. f. physiol. Chem. 1904. XLII. 554.

Buchner, Ed., u. Rapp, R.,
I. Alkoholische Gärung ohne Hefezellen.—Ber. d. D. Chem. Ges. 1897. XXX. 2668.—C. f. B. 2. Abt. 1898. IV. 297.—Ch. C. 1898. I. 70.
II. *Ibid.* II.—Ber. d. D. Chem. Ges. 1898. XXXI. 209. — C. f. B. 2. Abt. 1898. IV. 522.—Ch. C. 1898. I. 681.—K. J. IX. 209.

III. *Ibid.* V. (should be III.) Mittlg.—Ber. d. D. Chem. Ges. 1898. XXXI. 1084. —C. f. B. 2. Abt. 1898. IV. 927.—Ch. C. 1898. II. 47.—K. J. IX. 312.
IV. *Ibid.* VI. (should be IV.) Mittlg.—Ber. d. D. Chem. Ges. 1898. XXXI. 1090.—C. f. B. 2. Abt. 1898. IV. 860. —Ch. C. 1898. II. 48.—K. J. IX. 312.
V. *Ibid.* VII. (should be V.) Mittlg.—Ber. d. D. Chem. Ges. 1898. XXXI. 1531. —Ch. C. 1898. II. 438. — K. J. IX. 314.
VI. *Ibid.* VIII. (VI.).—Ber. d. D. Chem. Ges. 1899. XXXII. 127.—C. f. B. 2. Abt. 1899. V. 312.—Ch. C. 1899. I. 531.—K. J. X. 313.
VII. *Ibid.* IX. (VII.).—Ber. d. D. Chem. Ges. 1899. XXXII. 2086.—C. f. B. 2. Abt. 1899. V. 843.—Ch. C. 1899. II. 446.—K. J. X. 317.
VIII. *Ibid.* X. (VIII.).—Ber. d. D. Chem. Ges. 1901. XXXIV. 1523.—C. f. B. 2. Abt. 1901. VII. 809. — Ch. C. 1901. II. 140.
IX. Ber. d. D. Chem. Ges. 1898. XXXI. 1085.
X. Ber. d. D. Chem. Ges. 1901. XXXIV. 1527.
XI. Ber. d. D. Chem. Ges. 1898. XXXI. 2671.
XII. Ber. d. D. Chem. Ges. 1897. XXXV. 2668.

Buchner, Ed., und Spitta, Alb.,
I. Zymasebildung in der Hefe.—Ber. d. D. Chem. Ges. 1902. XXXV. 1703. —Ch. C. 1902. I. 1371.

Buchner, Hans,
I. Über die vermeintlichen Sporen des Typhusbacillus.—C. f. B. 1888. IV. 353.
II. Über die experimentelle Erzeugung des Milzbrandcontagiums aus den Heupilzen. — Nägeli's Untersuchungen über niedere Pilze. München. 1882. p. 140.
III. Kritisches und Experimentelles über die Frage der Konstanz der pathogenen Spaltpilze. — Nägeli's Untersuchungen, etc. p. 272.
IV. Über den Einfluss des Lichtes auf Bakterien.—C. f. B. 1892. XI. 781; XII. 217.—K. J. III. 76.
V. Über den Einfluss des Lichtes auf Bakterien und über die Selbstreinigung der Flüsse.—Archiv f. Hygiene. 1894. XVII. 177.—C. f. B. 1894. XV. 515.
VI. Beiträge zur Morphologie der Spaltpilze.—Nägeli's Untersuchungen, etc. S. 205.
VII. Eine neue Methode zur Kultur anaerober Mikroorganismen.—C. f. B. 1888. IV. 149.
VIII. Notiz betreffend die Frage des Vorkommens von Bakterien im normalen Pflanzengewebe.—Münch. Medic. Wochenschrift. 1888. XXXV. 906.—C. f. B. 1889. V. 341.—Z. g. Br. 1889. XII. 21.

Buchner, Hans, und Gruber, Max,
I. Verfahren zur Gewinnung von Hefeneiweiss mittelst Äther behufs Verwendung als Nährmittel.—German Pat. No. 113181, May 24, 1899.—Ch. C. 1900. II. 829.

Buchner, H., Longard, K., u. Riedlin, G.,
I. Über die Vermehrungsgeschwindigkeit der Bakterien.—C. f. B. 1887. II. 1.

Buchner, H., und Rapp, Rudolf,
I. Beziehungen des Sauerstoffes zur Gärtätigkeit der lebenden Hefezellen.—Zeitschrift f. Biologie. 1898. XXXVII. 82.—Ch. C. 1899. I. 133.—K. J. IX. 77.

Buchner, H., und Segall,
I. Über gasförmige antiseptische-Wirkungen des Chloroformes, Formaldehydes und Kreolins.—Münchner medic. Wochenschrift. 1889. No. 20.—Ch. C. 1889. II. 460.

Bucholz, W.,
I. Pharmac. Zeitschr. f. Rüssland. 1867. 627, 686.

Büchseler, Max,
I. Beiträge zur Beurteilung der Reinhefe Rasse II.—Z. f. Spiritusind. 1894. XVII. No. 8.—K. J. V. 176.

Büsgen, Moritz,
I. Kulturversuche mit Cladothrix dichotoma.—Ber. d. D. Bot. Ges. 1894. XII. 147.—C. f. B. 1894. XVI. 860.—K. J. V. 57.
II. Über einige Eigenschaften der Keimlinge parasitischer Pilze.—Bot. Ztg. I. Abt. 1893. LI. 53. Gives the prior literature relating to irritability.
III. Die Entwicklung der Phycomyceten-Sporangien. Dissert. Strassburg. 1882. —Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1882. XIII. 253.
IV. Ber. d. D. Bot. Ges. 1885. 66.

Bütschli, O.,
I. Über den Bau der Bakterien und verwandter Organismen. Vortrag. Leipzig. 1890. Winter.—Bot. Ztg. 1890. XXXXVIII. 463.—C. f. B. 1890. VII. 639.
II. Untersuchungen über mikroskopische Schäume und das Protoplasma. Leipzig. 1892. Engelmann.—C. f. B. 1893. XIII. 436.—K. J. IV. 54.

Bujwid, Odo,
I. Die Bakterien in Hagelkörnern.—C. f. B. 1888. III. 1.

Bulling, A.,
I. Spontane Lungentuberkulose einer Ziege.—Münchener medizin. Wochenschrift. 1896. p. 474.—Baumgarten's Jahresbericht. XII. 465.

Bungener,
I. Moniteur Scientifique. 1882. XXIV. 523, 829.

Bungener, H., und Weibel, L.,
I. Einges über die Zusammensetzung des Würze-Extraktes.—Allgem. Brauer- und Hopfen-Zeitung. 1891. p. 65.—Z. g. Br. 1891. XIV. 57.
II. Z. g. Br. 1891. XIV. 75.

Burci, E., e Frascani, V.,
I. Contributo allo studio dell' azione battericido della corrente continua.—Atti della Soc. Tosc. di Scienze nat. Mem. Vol. XII. 1891.—K. J. III. 76.

Burdon-Sanderson,
I. The Origin and Distribution of Microzymes (Bacteria) in Water, and the Circumstances which determine their Existence in the Tissues and Liquids of the Living Body.—Quarterly Journal of the Microscopical Society. 1871. XI.

Burgerstein, Alfred,
I. Untersuchungen über das Vorkommen und die Entstehung des Holzstoffes in den Geweben der Pflanzen. — Sitzgs.-Ber. d. Wiener Akademie. 1874. 1. Abt. LXX. 338. — Gives the antecedent literature.

Burkard u. Seifert,
I. Pharmac. Centralhalle. 1895. XXXVI. 365.

Burri, Robert,
I. Über einen milzbrandähnlichen Bacillus aus südamerikanischem Fleischfuttermehl.— Hygienische Rundschau. 1894. IV. No. 8.—C. f. B. 1894. XVI. 374.

Burri, R., Herfeldt, E., und Stutzer, A.,
I. Bakteriologisch-chemische Forschungen über die Ursachen der Stickstoff-Verluste in faulenden organischen Stoffen insbesondere im Stallmist und in der Jauche. —Journal für Landwirtschaft. 1894. XLII. 329.—C. f. B. 2. Abt. 1895. I. 284.

Burri, R., und Stutzer, A.,
I. Über einen interessanten Fall einer Mischkultur.—C. f. B. 1894. XVI. 815. —Ch. C. 1895. I. 96 —K. J. V. 88.
II. Über Nitrat zerstörende Bakterien und den durch dieselben bedingten Stickstoffverlust.—C. f. B. 2. Abt. 1895. I. 257; 1896. II. 473.—Ch. C. 1895. II. 571, 638.—K. J. VI. 285.
III. Über einen auf Nährgelatine gedeihenden nitratbildenden Bacillus.—C f. B. 2. Abt. 1895. I. 721; 1896. II. 105. —Ch. C. 1896. I. 267, 1011. —K. J. VI. 278.

Burstert, H., und Herz, F. J.,
I. Rote Käse.—Mitteilungen des Milchwirtsch. Vereins im Allgäu. 1895. No. 6.—Chemikerzeitung Repertorium. 1895. XIX. 274.

Buscaglioni,
See Fermi und Buscaglioni.

Buscalioni, L.,
I. Il Saccharomyces guttulatus.—Malpighia. 1896. X. 281.—K. J. VII. 29.

Buscalioni, L., e Casagrandi, O.,
I. Sul Saccharomyces guttulatus (Rob.) nuove osservazioni.—Malpighia. 1898. XII. 59.—C. f. B. 2. Abt. 1899. V. 311.

Busse, Otto,
I. Die Hefen als Krankheitserreger. Berlin. 1897.—C. f. B. 1. Abt. 1897. XXII. 349.

Butkewitsch, Wl.,
I. Über das Vorkommen proteolytischer Enzyme in gekeimten Samen und über ihre Wirkung.—Ber. d. D. Bot. Ges. 1900. XVIII. 185.—Ch. C. 1900. II. 386.
II. Jahrb. Wissensch. Bot. 1903. XXXVIII. 147.

Butlerow,
I. Liebig's Ann. 1868. CXLV. 277.

Buttenberg, Paul,
See Abel und Buttenberg.

Buxton, B. H.,
I. American Medicine. 1903. V. 137. Abst. in W. f. Br. 1904. XXI. 722.

Cagniard-Latour, Charles,
I. Sur la fermentation vineuse.—L'Institut. Nos. 158, 159, 164, 165, 166, 167, 185, 199.—Journal für prakt. Chemie. 1836. VIII. 415.
II. Mémoire sur la fermentation vineuse. —C. R. 1837. IV. 905.—More detailed in Annales de Chimie et de Physique. 1838. LXVIII. 206. — Journal für prakt. Chemie. 1839. XVI. 347.

Cahours,
I. Ann. de chim. et de phys. 1839. LXX. 81.

Cahours et Demarçay,
I. C. R. 1875. LXXX. 1568.

Calmette, A.,
I. Contribution à l'étude des ferments d'amidon. La levure chinoise. — Ann. Inst. Past. 1892. VI. 604.—C. f. B. 1. Abt. 1893. XIII. 273.—Ch. C. 1893. II. 86.—K. J. III. 111.
II. Revue Scientif. 1892. Feb. 27.

Cambier, R.,
I. Contribution à l'étude de la fermentation ammoniacale et des ferments de l'urée.—Annales de micrographie. 1893. V. 322.—K. J. IV. 285.

Cambier, R., et Brochet, A.,
I. Sur la production de l'aldéhyde formique gazeux destiné à la désinfection.—C. R. 1894. CXIX. 607. — Ch. C. 1894 II. 857.—K. J. V. 96.

Campbell, George F.,
See Osborne und Campbell.

Camus, L.,
I. Formation de lipase par le Penicillium glaucum.—Comptes rendus de la Société de Biologie. 1893. XXXXIX. 192.—K. J. VIII. 269.

II. De la lipase dans les cultures d'Aspergillus niger.—*Ibid.* p. 230.—K. J. VIII. 269.
III. Comptes rendus de Soc. de Biol. 1897. XLIX. 192.
IV. *Ibid.* p. 230.

CANDOLLE, Augustin Pyramus de,
I. Mémoires sur la famille des Légumineuses. Paris. 1825, p. 22.

CARLES, P.,
I. Über das Umschlagen der Weine.—Biedermann's Centralblatt. 1883. XII. 790; from Répertoire de pharmacie et journal de chimie médicale. 1883. XI. 59.
II. Sur la caracteristique des vins de figue.—C. R. 1891. CXII. 811.

CARO, O.,
I. Della maniera in cui i bacilli del carbonchio si comportano nel latte nelle prime 24 ore.—La Riforma med. 1893. p. 84.—C. f. B. 1893. XIV. 398.—Ch. C. 1894. I. 164.

CASAGRANDI, O.,
I. Sulla morfologia dei Blastomiceti.—Naturalista Siciliano. 1897. No. 1 a 3.—German translation in C. f. B. 2. Abt. 1897. III. 563.

CASPARI, Theodor,
I. Über zwei- und dreierlei Früchte einiger Schimmelpilze (Hyphomyceten).—Monatsberichte d. Kgl. Akademie d. Wissenschaften. Berlin. 1855. p. 308.—Flora. 1855. XXXVIII. 483.

CATHCART, E., u. M. HAHN,
I. Archiv. f. Hyg. 1902. XLIV. 295.

CATHELINEAU, H., und LEBRASSEUR, A.,
I. Über Anwendung der Fluoride in den Gärungsgewerben und über ihre toxische, therapeutische und physiologische Wirkung.—Revue internationale des falsifications, etc. 1895. VIII. 70.—Ch. C. 1895. I. 306.—Gives information relative to the principal works of Effront and others on the action of fluorides.

CATTANI, Giuseppina,
See TIZZONI e CATTANI.

CAZENEUVE, P., et HADDON,
I. Sur les causes de la coloration et de la coagulation du lait par la chaleur.—C. R. 1895. CXX. 1272.—Ch. C. 1895. II. 231.

CECH, C. O.,
I. Über den Ursprung der Hopfenkultur.—Z. g. Br. 1881. IV. 277.

CELLI, A.,
I. Die Kultur der Amöben auf festem Substrate.—C. f. B. 1. Abt. 1896. XIX. 536.

CERKEZ, S. C.,
I. Über Braga.—Zeitschrift f. Untersuchung d. Nahrungs- und Genussmittel. 1899. II. 29.—Ch. C. 1899. I. 572.—K. J. X. 171.

ČERNY, Franz,
I. Über die Gärung der Bierwürze bei verschieden grossen Mengen des beigemengten Trubes.—Österr. Brauer- und Hopfenzeitg. 1895. VIII. 277.—Z. g. Br. 1896. XIX. 112.—K. J. VI. 162.
II. Über die Hefengabe und ihren Einfluss auf die Gärung.—Österr. Brauer- und Hopfenzeitg. 1895. VIII. 225.—Z. g. Br. 1896. XIX. 100.—K. J. VI. 141.

CHAMBERLAND, Ch.,
I. Recherches sur l'origine et le développement des organismes microscopiques. Thèse. Paris. 1879.
See also PASTEUR, JOUBERT et CHAMBERLAND.

CHANCEL,
I. C. R. 1853. XXXVIII. 410.

CHAPMAN, C.,
I. The Volatile Bye-products of Fermentation.—Journal of the Federated Institutes of Brewing. 1897. III. 240.—Ch. C. 1898. I. 72.—K. J. VIII. 101.

CHARPENTIER,
I. C. R. 1905. CXLI. 367; 429.

CHARRIN, A., et PHISALIX,
I. Abolition persistante de la fonction chromogène du Bacillus pyocyaneus.—C. R. 1892. CXIV. 1565.—C. f. B. 1893. XIV. 429.—K. J. III. 88.
See also D'ARSONVAL et CHARRIN.

CHESHIRE, Frank R., and CHEYNE, W. Watson,
I. The Pathogenic History and History under Cultivation of a New Bacillus (B. alvei), the Cause of a Disease of the Hive Bee hitherto known as Foul Brood.—Journal of the Royal Microscop. Society. Ser. II. 1885. V. 381.
See also F. C. HARRISON (I.).

CHEVALLIER,
I. Note sur le pain moisi.—Annales d'Hygiene publique et de médecine légale. 1843. XXIX. 39.

CHEYNE, W. Watson,
See CHESHIRE and CHEYNE.

CHIAROMONTE, Tom.,
See FONSECA und CHIAROMONTE.

CHICANDARD, G.,
I. Sur la fermentation panaire.—C. R. 1883. LXXXXVI. 1585; LXXXXVII. 616.—Ch. C. 1883. pp. 439, 641.

CHODAT und BACH,
I. Ber. d. D. Chem. Ges. 1902. XXXV. 1275.

CHRISTMAS,
I. Sur la valeur antiseptique de l'ozone.—Ann. Inst. Past. 1893. VII. 776.—C. f. B. 1894. XV. 1016.—K. J. IV. 110.

CHRZASZCZ, T.,
I. Die Chinesische Hefe.—C. f. B. 2. Abt. 1901. VII. 326, 913.
II. C. f. B. 2. Abt. 1901. VII. 326.

CHUARD, E.,
I. Essai de vinification avec les levures selectionnées.—Extract from the Chronique agricole du canton de Vaud. 1892.—K. J. III. 145.

CHUARD, E., und JACCARD, M.,
I. Veränderungen der schwefligen Säure in den Weinen.—Ch. C. 1894. II. 129.

CHUDIAKOW, N. von,
I. Untersuchungen über die alkoholische Gärung. — Landw. Jährbücher. 1894. XXIII. 391.—C. f. B. 2. Abt. 1895. I. 122, 188.—Bot. Ztg. 2. Abt. 1894. LII. 257, 289.—Ch. C. 1894. II. 291.—K. J. V. 123.

CIENKOWSKI, L.,
I. Die Gallertbildungen des Zuckerrübensaftes. Charkow. 1878.—German abstract in Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1878. p. 1017. Gives the plate.
II. Die Pilze der Kahmhaut.—Biological Selections from the Bulletin de l'Académie imp. des sciences de St. Pétersbourg. 1872. VIII. 566.
III. Bull. de l'Acad. imp. de St. Pétersbourg. 1872. XVII. 513.

CLAESSEN, Heinrich,
I. Über einen indigoblauen Farbstoff erzeugenden Bacillus aus Wasser.—C. f. B. 1890. VII. 13.

CLAUDON, Edouard, et MORIN, E. Ch.,
I. Produits de fermentation du sucre par la levure elliptique.—C. R. 1887. CIV. 1109.—C. f. B. 1. Abt. 1887. II. 655.
II. Sur la présence de l'alcohol butylique normal dans une eau-de-vie de Cognac.—C. R. 1887. CIV. 1187.

CLAUSSEN, N. H.,
I. W. f. Brauerei. 1904. XXI. 370.

CLAUTRIAU, M.,
I. Etude chimique du Glycogène chez les Champignons et les Levures.—Mémoires couronnés and other Mémoires published by the Acad. Roy. des Sciences, des Lettres et des Beaux-Arts de Belgique. Collection in 8°. 1895–1896. LII. 1.—C. f. B. 2. Abt. 1896. II. 429.—Z. g. Br. 1897. XX. 232, 245.—K. J. VI. 51.

CLERFEYT, Charles,
I. Expériences sur l'accoutumance héréditaire des Levures aux solutions salines concentrées.—Bulletin de l'Académie Royale de Belgique. Classe des Sciences. 1901. p. 337.

CNOPF, R. Th.,
I. Quantitative Spaltpilzuntersuchungen in der Kuhmilch.—Tageblatt der 62. Versammlung D. Naturforscher und Ärzte in Heidelberg. 1889. p. 494.—C. f. B. 1889. VI. 553.

COATES, Charles E., and DODSON, W. R.,
I. Stickstoffassimilation der Baumwollpflanze.—Journal of the Amer. Chem. Soc. 1896. XVIII. 428.—Ch. C. 1896. II. 45.—K. J. VII. 206.

COHN, Ferdinand,
I. Untersuchungen über Bakterien. I.—Beiträge zur Biologie der Pflanzen. Bd. I., Heft 2, p. 127. Breslau. 1870 bis 1875.
II. *Ibid.* II.—*Ibid.* Bd. I. Heft 3. p. 141.
III. *Ibid.* III.—*Ibid.* p. 208.
IV. Versuch eines natürlichen Systems der Kryptogamen.—Hedwigia. 1872. No. 1.
V. Über die Entwicklungsgeschichte mikroskopischer Algen und Pilze.—Nova acta Academiae Carol. Leop. nat. cur. 1853. XXIV. I. 123.
VI. Über Bakterien, die kleinsten lebenden Wesen.—Virchow und Holtzendorff's Sammlung gemeinverst. Vorträge. 7. Serie. Heft 165. Berlin. 1872.
VII. Untersuchungen über Bakterien. IV.—Beiträge zur Biologie der Pflanzen. 1877. Bd. II. Heft 2. p. 249.
VIII. Über thermogene Bakterien.—Ber. d. D. Bot. Ges. 1893. XI. 66.—C. f. B. 1893. XV. 424.—K. J. IV. 81.
IX. Über Wärmeerzeugung durch Schimmelpilze und Bakterien. Vortrag. Breslau. 1890.—K. J. I. 40.
X. Über thermogene Wirkung von Pilzen. —Jahresbericht d. Schlesisch. Ges. f. vaterländ. Kultur. 1888. p. 150.—C. f. B. 1889. VI. 351.—K. J. I. 40.
XI. Über den Brunnenfaden (Crenothrix polyspora).—Beiträge zur Biologie der Pflanzen. 1870. I. Band. I. Heft. p. 108.
XII. Über zwei neue Beggiatoen.—Hedwigia. 1865. p. 81.
XIII. Jahresb. d. Schles. Ges. f. vaterländsche Kultur. 1883. Breslau. 1884. 226.
XIV. *Ibid.* 1888. 156.
XV. *Ibid.* 1890.
XVI. *Ibid.* 1883. LXI. 226.

COHN, F., und MENDELSOHN, B.,
I. Über Einwirkung des elektrischen Stromes auf die Vermehrung von Bakterien.—Beiträge zur Biologie der Pflanzen. 1879. III. 141.

COHNHEIM,
I. Z. f. physiol. Chemie. 1903. XXXIX. 336.

COLIN,
I. Ann. d. chim. et de physique. 1824. XXVII. 128; XXX. 42.

COLLETTE, August, und BOIDIN, August,
I. Verfahren zur Gewinnung von Alkohol aus stärkehaltigem Material unter Benutzung aseptischer Verzuckerung und Vergärung mittelst Mucedineen.—German Patent No. 99253, Aug. 17, 1897. —Z. f. Spiritusind. 1899. XXI. Ergänzungsheft I. 63.—Ch. C. 1899. I. 155.—K. J. VIII. 262.
II. Zusatz-Patent zu I.—German Patent No. 100129, Jan. 28, 1898.—Z. f. Spiritusind. 1899. XXI. Ergänzungsheft. I. 64.—Ch. C. 1899. I. 576.
III. Engl. Patent No. 13053, June 10, 1898.—K. J. IX. 288.
IV. Engl. Patent No. 19858, Aug. 28, 1897.—K. J. IX. 288.
V. Engl. Patent No. 1155, Jan. 15, 1897. —K. J. IX. 287.

CONN, H. W.,
I. Cream Ripening with Bacillus No. 41.—C. f. B. 2. Abt. 1895. I. 385.
II. The Relation of Pure Cultures to the Acid, Flavour and Aroma of Butter.—C. f. B. 2. Abt. 1896. II. 409.—Ch. C. 1896. II. 636.—K. J. VII. 182.
III. The Isolation of Rennet from Bacteria Cultures.—Fifth Report of the Storrs Agricultural School of the Experiment Station, Middletown, Connecticut. 1892, p. 106.—C. f. B. 1892. XVI. 914.—K. J. III. 259.
IV. Über einen bittere Milch erzeugenden Micrococcus.—C. f. B. 1891. IX. 654.—K. J. II. 185.

CONN, THOM, BOSWORTH, STOCKING, and ISSAJEFF,
I. (Chap. 56) Storrs Agric. Exp. Station, Storrs, Conn. 1905. Bull. No. 35.
II. (Chap. 57) U.S. Dept. of Agriculture. Bull. No. 71. Bureau of Animal Industries, Washington. 1905.

CORDIER, J. A.,
I. Contribution à la biologie des levures de vin.—C. R. 1898. CXXVII. 628.—C. f. B. 2. Abt. 1899. V. 105.

CORNU, MAX,
I. Etudes sur le Phylloxera. Paris. 1878, p. 159.

COSSETTINI, G.,
I. Bolletino chim. farm. 1901. XL. 75.

COSTANTIN, J.,
I. Recherches sur le Cladosporium herbarum.—Journal de Botanique. 1889.
II. Sur les variations des Alternaria et Cladosporium.—Revue générale de Botanique. 1889.
III. Les mucédinées simples. Paris. 1888. P. Klincksieck. With 190 figures.
IV. Bull. Soc. Bot. de France. 1893. 236.

COSTANTIN et LUCET,
I. Bull. Soc. Mycol. de France. 1903. XIX. 33.
II. Ann. d. Sci. Nat. Bot. 1905. 9th Series. II. 119.

COSTANTIN et RAY,
I. C. R. de la Soc. de Biologie. 1898. 10th Series. V. 504.

COUPIN,
I. C. R. 1904. CXXXVIII. 389.

COUPIN et FRIEDEL,
I. C. R. 1904. CXXXVIII. 1118.

CRAMER, E.,
I. Die Zusammensetzung der Bakterien in ihrer Abhängigkeit vom Nährmaterial.—Habilitationsschrift, Heidelberg.—A. f. Hygiene. 1892. XVI. 151.—C. f. B. 1. Abt. 1893. XIV. 12.—Ch. C. 1893. I. 543.—K. J. IV. 67.
II. Die Zusammensetzung der Cholerabacillen.—A. f. Hygiene. 1895. XXII. 167.—C. f. B. 1. Abt. 1895. XVII. 611.—Ch. C. 1895. I. 850.
III. Die Zusammensetzung der Sporen von Penicillium glaucum und ihre Beziehung zu der Widerstandsfähigkeit derselben gegen äussere Einflüsse.—A. f. Hygiene. 1894. XX. 197.—C. f. B. 2. Abt. 1895. I. 499.—Ch. C. 1894. II. 54.—K. J. V. 86.
IV. Die Ursache der Resistenz der Sporen gegen trockene Hitze.—A. f. Hygiene. 1891. XIII. 71.—C. f. B. 1. Abt. 1892. XI. 451.—Ch. C. 1891. II. 760.—K. J. II. 85.

CRAMER, Carl,
I. Quoted by KABSCH (I.) from the Vierteljahrsschrift d. naturforsch. Ges. in Zürich. 1858. III. 1.
II. Vierteljahr. d. naturf. Ges. in Zürich. 1859. 325.

CREMER, MAX,
I. Über die Umlagerung der Zuckerarten unter dem Einflusse von Ferment und Zelle.—Zeitschr. f. Biologie. 1894. XXXI. 183.—Ch. C. 1894. II. 245.—K. J. V. 112.
II. Das Verhalten einiger Zuckerarten im tierischen Organismus. — Zeitschr. f. Biologie. 1893. XXIX. 484.—Ch. C. 1893. II. 693.
III. Demonstration des Hefeglycogens in den Zellen.—Münchener med. Wochenschrift. 1894. No. 26.—Ch. C. 1894. II. 245.—K. J. V. 113.
IV. Über Hefe und Leberzelle.—Münchener med. Wochenschrift. 1894.—Ch. C. 1894. II. 245.—K. J. V. 113.
V. Zucker und Zelle.—Zeitschr. f. Biologie 1895. XXXII. 1.—K. J. VI. 55.
VI. Über Glycogenbildung im Hefepresssaft.—Ber. d. D. Chem. Ges. 1899. XXXII. 2062.—C. f. B. 2. Abt. 1900. VI. 90.—Ch. C. 1899. II. 447.
VII. Sitz. d. Ges. f. Morphologie u. Physiologie München. 1894. Hft. 1.

CROCHETELLE, J.,
See DUMONT et CROCHETELLE.

CROSS, C. F., and BEVAN, E. J.,
I. Die Kohlenhydrate der Gerste.—Journa of the Federated Institutes of Brewing. 1897. III. 2.—Ch. C. 1897. II. 1028.

CROSS, C. F., BEVAN, E. J., and SMITH, Claud,
I. Über einige chemische Vorgänge in der Gerstenpflanze.—Ber. d. D. Chem. Ges. 1895. XXVIII. 2604.—Ch. C. 1895. II. 1129.

CROUZEL,
I. Schwefelwasserstoff bildende Hefe.—L'union pharmac. 1891. T. 32. 1892. T. 33, p. 60.—C. f. B. 1892. XI. 800.—K. J. II. 135; III. 119.
II. Journ. de pharm. et chimie. 1891. (5.) XXIII. 309.

CUBONI, G.,
I. Über die durch Botrytis cinerea bedingte Fäulnis der Rebentriebe.—Bollettino di Notizie agrarie. 1896. II. 487.—C. f. B. 2. Abt. 1897. III. 330.

II. Sulla probabile origine dei Saccaromiceti.—Rivista di viticoltura ed enologia italiana. 1885. IX. No. 12–13. —Just's Botan. Jahresbericht pro 1885. I. 277.

Cugini, G.,
I. Sulla alimentazione delle piante cellulari.—Nuovo giornale botan. Ital. 1876. VIII. 77, 261.—Just's Bot. Jahr. pro 1876. p. 113.

Cuisinier, Léon,
I. Une nouvelle matière sucrée diastasique la Céréalose et pour sa fabrication. French Patent No. 171958.—La sucrerie indigène et coloniale. 1886. XXVII. 241.—C. f. B. 2. Abt. 1895. I. 329.—Ch. C. 1887. p. 614.

Curtis,
I. Contribution à l'étude de la Saccharomycose humaine.—Ann. Inst. Past. 1896. X. 449.

Czapek, Friedrich,
I. Über Orseillegärung.—C. f. B. 2. Abt. 1898. IV. 49.—Ch. C. 1898. I. 684.
II. Zur Biologie der holzbewohnenden Pilze.—Ber. d. D. Bot. Ges. 1899. XVI. 166.—C. f. B. 2. Abt. 1899. V. 872.
III. Beitr. z. Chem. Phys. u. Path. 1902. I. 538; II. 557; III. 62.—Ber. d. D. Bot. Ges. 1902. XIX. 130.
IV. Biochem. d. Pflanz. Jena, 1905.
V. Ber. d. D. Bot. Ges. 1899. XVII. 166.

Czaplewski, E.,
I. Zur Anlage bakteriologischer Museen.—C. f. B. 1889. VI. 409.

Czéh, Andreas, u. Müller-Thurgau, H.,
I. Welche Vorgänge finden während der Gärung und der Weiterentwicklung des Weines statt?—Weinbau und Weinhandel. 1888. VI. 121.

Dachnjewski,
I. Vergleichende Wertprüfung der Filter von Chamberland und von Berkefeld.—Wratsch. 1893. No. 13.—C. f. B. 1894. XVI. 664.—K. J. V. 19.

Dahlen, H. W.,
I. Neuere Beobachtungen über den sogen. Edelfäulepilz (Botrytis cinerea).—Weinbau und Weinhandel. 1894. XII. 306.

Dahmen, Max,
I. Die bakteriologische Wasseruntersuchung.—Chemikerzeitung. 1892. XVI. 861.—C. f. B. 1892. XII. 302, 620.

Dangeard, P. A.
I. Observations sur le groupe des bactéries vertes.—Annales de micrographie. 1895. V. 67.
II. Sur la structure histologique des levures et leur développement.—C. R. 1893. CXVII. 68.—K. J. IV. 51.
III. C. R. 1908. CXXXVII. 1281.

Danilewsky, A., u. Radenhausen, P.
I. Zur chemischen Konstitution der Milch. —Schweiz. Zeitschrift f. Pharmacie. 1880. No. 22.—Ch. C. 1880. p. 519.

Le Dantec, Felix,
I. Etude de la morue rouge.—Ann. Inst. Past. 1891. V. 636.—C. f. B. 1892. XI. 198.—K. J. II. 234.

Darexy, Prosper,
I. Recherches sur la matière grasse de la levure de bière.—Toulouse. 1896. Marquès & Co. 47 pp.
See also Gérard et Darexy.

Dastre,
I. C. R. 1883 XCVI. 932.

Davaine.
I. Recherches sur les Vibrioniens.—C. R. 1864. LIX. 393, 629.

Dean.
I. Botan. Gazette. 1903. XXXV. Jan.

Decaisne, E.,
I. Sur l'oïdium aurantiacum du pain.—C. R. 1871. LXXIII. 507.
II. Sur l'oïdium aurantiacum du pain au point de vue pathologique.—C. R. 1871. LXXIII. 684.

Dehérain, P. P.,
I. Sur la fabrication du fumier de ferme. —C. R. 1884. LXXXXVIII. 377; LXXXXIX. 45.—Ch. C. 1884. pp. 262, 709.
II. Über die Buttersäuregärung in den Diffusionsgefässen der Zuckerfabriken. —Zeitschrift d. Vereins f. d. Rübenzucker-Industrie. 1884. XXI. 269.—Ch C. 1884. p. 403.
III. Sur la composition des eaux de drainage d'hiver des terres nues et emblavées. —C. R. 1893. CXVII. 1041.—K. J. IV. 235.
IV. Le travail de la terre et la nitrification.—C. R. 1893. CXVI. 1091.—K. J. IV. 234.

Delacroix, E.,
I. Darstellung von Milchsäure aus Milchserum.—Journal de Pharmacie et de Chimie. 1891. (5.) XXIII. 287.—Ch. C. 1891. I. 698.—K. J. II. 178.
II. Bull. Soc. Mycol. de France. 1891. VII. 107.

Delbrück, Max,
I. Grundlagen für ein Preisausschreiben zur Lösung der Schaumgärungs-Frage.—Z. f. Spiritusind. 1892, 1893.—K. J. IV. 155, 157.
II. Fortschritte im Arbeiten mit Reinhefe. —Z. f. Spiritusind. 1895. XVIII. Ergänzungsheft. p. 25.—K. J. VI. 178.
III. Das Pilzmaischverfahren. — Z. f. Spiritusind. 1899. XXII. Ergänzungsheft. II. 52.
IV. Die Bewegung des Stickstoffs in der gärenden Maische als Massstab der Gärungsführung. — Z. f. Spiritusind. 1879. II. 310, 351.
V. Über die physiologische Methode der Eiweissbestimmung für Würze und Bier und ihre praktische Bedeutung.—W. f. Br. 1893. X. 810.—Ch. C. 1894. I. 846.—K. J. IV. 139.

VI. Der Einfluss der Lüftung auf Hefe und Gärung und ihre Benutzung zur Vermehrung der Hefeausbeute in der Presshefefabrikation und zur Vergärung der Dickmaischen.—Z. f. Spiritusind. 1890. XIII. Ergänzungsheft. p. 31.—K. J. I. 59.
VII. Alkoholische Gärung ohne Hefezellen.—W. f. Br. 1897. XIV. 363.—Ch. C. 1898. I. 70.—K. J. VIII. 307.
VIII. Die Carlsberger reingezüchtete Hefe.—W. f. Br. 1885. II. 126.
IX. W. f. Br. 1890. VII. 636.
X. *Ibid.* 1898. XV. 650.
XI. *Ibid.* 1902. XIX. 25.
XII. *Ibid.* 1903. XX. 66.
XIII. Chem. Zeitg. 1899. XXIII. 176.
See also Hayduck u. Delbrück.

Delffs,
I. Poggendorff. Annalen. 1851. LXXIV. 505.

Demme, R.,
I. C. f. Bakt. 1890. IX. 271.

Denayer, A.,
I. Engl. Patent No. 13032 of 1898.

Denys, J., et Martin, J.,
I. Sur les rapports du Pneumobacille de Friedländer, du ferment lactique et de quelques autres organismes avec le Bacillus aërogenes et le Bacillus typhosus.—La Cellule. 1893. IX. 261.—C. f. B. 1894. XVI. 127.

Deschamps,
I. Über das Lab.—Journal de Pharmacie. 1840, p. 412.—Dingler's polyt. Journal. 1840. LXXVIII. 445.

Desmazières,
I. Ann. d. Sci. Nat. Bot. 1834. 2nd Series. II. 71.

Destrem, A.,
See Schützenberger et Destrem.

Diakonow, N. W.,
I. La respiration intramoléculaire et la fermentation des champignons moisissures.—Archives slaves de Biologie. 1886. I. 531.—Ber. d. D. Bot. Ges. 1886. IV. 2.—Z. g. Br. 1886. IX. 153.
II. Sur le rôle de la substance nutritive fermentescible dans la vie de la cellule végétale.—Archives slaves de Biologie. 1887. IV. 31.—Ber. d. D. Bot. Ges. 1887. V. 380.—Z. g. Br. 1888. XI. 460.

Dickinson, W. F.,
See Lea and Dickinson.

Dierkx,
I. Ann. Soc. Scient. de Bruxelles. 1901. XXV.

Dieudonné, A.,
I. Beiträge zur Kenntnis der Anpassungsfähigkeit der Bakterien an ursprünglich ungünstige Temperaturverhältnisse.—Arbeiten aus dem Kaiserl. Gesundheitsamte. 1894. IX. 492.—C. f. B. 1894. XVI. 965.—K. J. V. 86.
II. Eine einfache Vorrichtung zur Erzeugung von strömenden Formaldehyd-Dämpfen für Desinfektionszwecke.—Arbeiten aus d. K. Gesundheitsamte. 1895. XI. 534.—Ch. C. 1895. II. 607.—K. J. VI. 15.

Dodson, W. R.,
See Coates and Dodson.

Domens, A.,
I. Keimgehalt ausschankreifer Flaschenbiere.—Allgem. Brauer- u. Hopfenzeitung. 1892. p. 2037.—Ch. C. 1893. II. 303.—K. J. IV. 141.

Dönitz, W.,
I. Über das Verhalten der Cholera vibrionen im Hühnerei.—Z. f. Hygiene. 1895. XX. 31.—Ch. C. 1895. II. 172.

Doepping, O.,
See Schlossberger und Doepping.

Dokkum, H.,
I. Über giftige Bestandteile von faulendem Käse.—Ch. C. 1894. II. 485.

Donath, E.,
I. Z. f. Naturwissenschaften. 1894. LXVII. 179.
II. Chem. Ztg. 1891. XV. 597.
III. Ber. d. D. Chem. Ges. 1875. VIII. 795.
IV. Chem. Ztg. 1892. XVI. 459.

Dormeyer, Carl,
I. Die rationelle Verwertung der Bierhefe.—W. f. Br. 1899. XVI. 557.—C. f. B. 2. Abt. 1900. VI. 375.—K. J. X. 162.
II. Verfahren zur Gewinnung der Eiweissstoffe aus Hefe. German Patent No. 111915, Apr. 18, 1899.—Ch. C. 1900. II. 607.

Dossios,
I. Jahresb. Chemie. 1866. 384.

Downes,
I. On the Action of Sunlight on Micro-organisms, with Demonstration of the Influence of Diffused Light.—Proceedings of the R. Society. 1886. XL. 14.

Downes and Blunt,
I. Researches on the Effect of Light upon Bacteria and other Organisms.—Proceedings of the R. Society. 1877. XXVI. 488.
II. On the Influence of Light on Protoplasma. — Proceedings, etc. 1878. XXVIII. 197, 199.

Dräer, Arthur,
See Abel und Dräer.

Dreyfuss, Isidor,
I. Über das Vorkommen von Cellulose in Bacillen, Schimmel- und anderen Pilzen.—Z. f. physiolog. Chemie. 1893. XVIII. 358.—C. f. B. 1894. XV. 909.—Ch. C. 1893. II. 941.—K. J. IV 73.

Drouin, R.,
See Gautier et Drouin.

Dubois, Raphael,
I. Extinction de la luminosité du Photobacterium sarcophilum par la lumière. —Comptes rendus de la Société de Biologie. 1893. II. 160.—K. J. IV. 128.
II. Sur le prétendu pouvoir digestif du liquide de l'urne des Népenthes.—C. R. 1890. CXI. 315.—K. J. I. 173.
III. C. R. 1890. CXI. 655.

Dubourg, E.,
I. De la fermentation des saccharides.—C. R. 1899. CXXVII. [? CXXVIII.] 440.—C. f. B. 2. Abt. 1899. V. 657.—Ch. C. 1899. I. 701.—K. J. X. 298.
II. Contribution à l'étude des levures de vin.—Revue de Viticulture. 1897, p. 467.—K. J. VIII. 100.
See also Gayon et Dubourg.

Dubrunfaut,
I. Sur la distillation des betteraves et la fermentation dite nitreuse.—C. R. 1868. LXVI. 275.—Ch. C. 1868. 941.
II. Mémoire sur la saccharification des fécules. Paris. 1882.
III. Sur la fermentation et le ferment alcooliques.—C. R. 1871. LXXIII. 263.
IV. C. R. 1856. XLII. 945.
V. *Ibid.* 1846. XXIII. 38. 1856. XLII. 902.

Duclaux, Emile Pierre,
I. Sur l'action antiseptique del' acide formique.—Ann. Inst. Past. 1892. VI. 593.—C. f. B. 1893. XIII. 75.—K. J. III. 74.
II. Sur les analogies entre les procès de fermentation et de combustion solaire. —Ann. Inst. Past. 1893. VII. 751.—C. f. B. 1894. XVI. 119.—K. J. IV. 85.
III. Action de l'Electricité sur les Microbes.—Ann. Inst. Past. 1890. IV. 677.
IV. Influence de la lumière du Soleil sur la vitalité des germes de microbes.—C. R. 1885. C. 119; CI. 395.—Ch. C. 1885. 166, 794.
V. Die Selbstreinigung der Flüsse.—Ann. Inst. Past. 1894. VIII. 117, 178.—Ch. C. 1894. I. 783, 1007.
VI. Sur le rôle protecteur des microbes dans la crème et les fromages.—Ann. Inst. Past. 1893. VII. 304.—K. J. IV. 208.
VII. Le lait. Etudes chimiques et microbiologiques. Paris. 1887. Ballière et fils.
VIII. Action de la présure sur le lait.—C. R. 1884. LXXXVIII. 526.—Ch. C. 1884. 343.
IX. Deuxième Mémoire sur le lait.—Annales de l'Institut national agronomique. 1883. VIII.
X. Traité de chimie biologique. Paris. 1883, p. 613.
XI. Nutrition sans bactéries.—Ann. Inst. Past. 1895. IX. 896.—Ch. C. 1896. II. 502.
XII. Sur le vieillissement des vins.—Ann. Inst. Past. 1893. VII. 537.—K. J. IV. 141.
XIII. Fabrication, maturation et maladies du fromage du Cantal. — Annales de l'Institut national agronomique. 1879. No. 5, p. 23.—Milchzeitung. 1879. 724, 740.
XIV. Fermentation du sucre de lait.—Ann. Inst. Past. 1887. I. 573.—C. f. B. 1. Abt. 1888. III. 525.
XV. Traité de microbiologie. Tome I. et II. 1899. Tome III. 1900.—C. f. B. 2. Abt. 1899. V. 773. 1900. VI. 255.
XVI. Sur la fermentation alcoolique.—C. R. 1864. LVIII. 1114; LIX. 450.
XVII. Sur l'absorption d'ammoniaque et la production d'acides gras volatiles pendant la fermentation alcoolique.—Annales de l'école normale supérieure. 1866. II.
XVIII. Pouvoir ferment et activité d'une levure.—Ann. Inst. Past. 1896. X. 119.—C. f. B. 2. Abt. 1896. II. 556.—Ch. C. 1896. I. 1169.—K. J. VII. 87.
XIX. Sur la conservation des levures.—Ann. Inst. Past. 1889. III. 375.—C. f. B. 1. Abt. 1889. VI. 412.
XX. Ann. Pasteur. 1892. 341.
XXI. Chimie biologique. 1883. 142, 193, 219.
XXII. Ann. Pasteur. 1889. III. 97.
XXIII. Ann. Pasteur. 1887. I. 573. 1889. III. 201.
XXIV. Traité de Microbiologie. Paris. 1898.
XXV. Journ. de la Brasserie. 1900.—Abst. in Chem. Centralbl. 1900. II. 54.
XXVI. Ann. Pasteur. 1897. XI. 348.
XXVII. Thèse. 1865.
XXVIII. C. R. 1874. LXXVIII. 1160.
XXIX. Traité de Microbiologie. II. 222.
XXX. Ann. Pasteur. 1889. III. 67.
XXXI. Thèse. 1865. 44.

Dujardin, Felix,
I. Histoire naturelle des Zoophytes. Paris. 1900.

Dujardin, J.,
I. Recherches rétrospectives sur l'art de la distillation. Historique de l'alcool, de l'alambic et de l'alcoométrie. Paris. 1900.

Düll, G.,
I. Zur Kenntnis des Bierextraktes.—Chemikerzeitung. 1892. XVI. 1178.—Ch. C. 1892. II. 764.
II. Chem. Beitg. 1895. XIX. 216.
III. Z. f. d. Ges. Brauwesen. 1894. XVII. 79.
See also Lintner und Düll.

Dumas, Jean Baptiste,
I. Traité de chimie appliquée aux arts. Paris. 1828–45.

II. Sur la conversion de l'ammoniaque en acide nitrique.—C. R. 1846. XX. 1020.
III. Recherches sur la fermentation alcoolique. 1872.
IV. Traité de chimie appliquée aux arts. VI. 316.
V. C. R. 1872. LXXV. 277.
VI. Ann. de chimie et de phys. 1874. 5th Series. III. 57.
VII. C. R. 1872. LXXV. 295.

DUMAS et BOULLAY,
I. Ann. de chimie et de phys. 1828. (2) XXXVII. 45.

DUMONT, J., et CROCHETELLE, J.,
I. Sur la nitrification des terres de prairie. C. R. 1893. CXVII. 670.—Ch. C. 1894. I. 97.—K. J. IV. 233.
II. Influence des sels de potassium sur la nitrification. — C. R. 1894. CXVIII. 604.—C. f. B. 2. Abt. 1895. I. 508.
III. De l'influence des chlorures sur la nitrification.—C. R. 1894. CXIX. 92. —Ch. C. 1894. II. 492.—K. J. V. 263.

DUNCKER, J.,
I. Die physikalische Prüfung der Desinfektion mit Wasserdampf. — Deutsche Medicinalzeitung. 1892. No. 85–91.—K. J. III. 27.

DÜNNENBERGER, Karl,
I. Bakteriologisch-chemische Untersuchung über die beim Aufgehen des Brotteiges wirkenden Ursachen.—Botan. Centralblatt. 1888. XXXIII. 245.—Archiv d. Pharmacie. 1888. XXVI. 544.—Ch. C. 1888. 667.

DUPETIT, G.,
See GAYON et DUPETIT.

DURIN, E.,
I. De la fermentation cellulosique du sucre de canne.—C. R. 1876. LXXXIII. 128. — Zeitschrift des Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1876. 752.
II. Bull. de l'assoc. des Chimistes. 1887. VI. 239.
III. *Ibid.* 1890. VIII. 296.

DURST, Otto,
I. Handbuch der Presshefe-Fabrikation. 2. Ed. Berlin. 1896. P. Parey. 16*s.* (bd.).

DUSCH, Th. von,
See SCHRÖDER u. DUSCH.

DUVAL, J.,
I. Nouveaux faits concernant la mutabilité des germes microscopiques.—Journal de l'anatomie et de physiologie. 1874. 489.

EBSTEIN, W.,
I. Einfluss der Kohlensäure auf die diastatischen Fermente.—Naturwissenschaftliche Rundschau. 1889. IV. 557. —Ch. C. 1889. II. 1028.

ECK,
I. Neue Zeitschr. f. deutsche Spiritusfabrikanten. 1877. XI. 149.

ECKENROTH, Hugo, und HEIMANN, R.,
I. Über Hefe und Schimmelpilze an den Trauben.—C. f. B. 2. Abt. 1895. I. 530. —Ch. C. 1895. II. 875.—K. J. VI. 39.

EFFRONT, Jean,
I. Action des acides minéraux dans la saccharification par le malt et la fermentation des matières amylacées.—Moniteur scientifique. 1890. IV. 449. —K. J. I. 72.
II. Etudes sur les levures. — Moniteur scientifique. 1891. V. 1137.—K. J. II. 156.
III. De l'influence des fluorures sur l'accroissement et le développement des cellules des levures alcooliques.—Moniteur scientifique. 1891. V. 254.—K. J. II. 154.
IV. Sur certaines conditions chimiques de l'action des levures de bière.—C. R. 1893. CXVII. 559.—K. J. IV. 164.
V. Nouvelle méthode pour la purification des levures.—Bulletin de la Société chimique de Paris. 1891. (3ème série.) V. 705.—Moniteur scientifique. 1891. V. 1137.—K. J. II. 156.
VI. Les Enzymes. Paris. Carré et Naud. I. Bd. 1899. 7*s.* 6*d.* German translation by M. Bücheler. Wien. 1899. 7*s.*—C. f. B. 2. Abt. 1900. VI. 176, 231.
VII. Studien über die Vergärbarkeit der Melasse.—Moniteur scientifique. 4e série. 1894. VIII. 161.—Ch. C. 1894. I. 786 —K. J. V. 155.
VIII. Action de l'oxygène sur la levure de bière.—C. R. 1898. CXXVII. 326. —Ch. C. 1898. II. 727.—K. J. IX. 79.
IX. Verfahren zur Gewöhnung von Hefe an die Dextringärung. German Patent No. 102631, April 3, 1898.—Engl. Patent No. 9615, April 26, 1898. — Z. f. Spiritusind. 1899. XXII. 126.—Ch. C. 1899. II. 500.—K. J. X. 110.
X. C. R. 1892. XCV. 1324.
XI. *Ibid.* 1894. CXIX. 92.
XII. *Ibid.* 1894. CXIX. 169.
XIII. *Ibid.* 1897. CXXV. 38, 116.
XIV. Bull. de l'Ass. des Chimistes de Sucrerie et Distillerie. 1905. XXIII. 393.
XV. Z. f. Spiritusindustrie. 1898. XXI. 298. 1899. XXII. 126.

EHLERS, T.,
I. Untersuchungen über den Rauschbrandpilz. Dissert. Rostock. 1884.

EHRENBERG, Alexander,
I. Experimentelle Untersuchungen über die Frage nach dem Freiwerden von gasförmigem Stickstoff bei Fäulnisprozessen. — Zeitschrift f. physiolog. Chemie. 1886. XI. 145, 438.—Ch. C. 1887. 15, 864.

EHRENBERG, Christian Gottfried,
I. Die Infusionstierchen als vollkommene Organismen. Leipzig. 1838.

EHRICH, E.,
I. Über die Darstellung schaumhaltiger und vollmundiger Biere.—Der Bierbrauer. 1897. 65.—K. J. VIII. 126.

EHRLICH,
I. Zeits. d. Vereins d. D. Zuckerindustrie. 1905. LV. 539.

EICHELBAUM, Georg,
I. German Patent No. 116127, Dec. 15, 1899.—Ch. C. 1901. I. 80.

EICHENGRÜN, A.,
I. Die neuen Arzneimittel im ersten Halbjahr 1899.—Zeitschrift f. angew. Chemie. 1899. 1147.—Ch. C. 1900. I. 144.

EIDAM, Eduard,
I. Die Einwirkung verschiedener Temperaturen und des Eintrocknens auf die Entwicklung von Bacterium Termo. —Cohn's Beiträge zur Biologie der Pflanzen. 1875. I. 3. Heft. 208. 1883. III. 128.
II. Über die Entwicklung von Sphaerotilus natans.—Jahresber. d. schlesischen Ges. f. vaterländ. Kultur. 1876.
III. Zur Kenntnis der Entwicklung bei den Ascomyceten.—Cohn's Beiträge zur Biologie der Pflanzen. 1883. III. 377.

EIJKMAN, C.,
I. Lichtgevende Bakterien. — Jaarverslag van het Labor. voor pathol. Anat. en Bact. te Weltevreden. 1891.—C. f. B. 1. Abt. 1892. XII. 656.—K. J. III. 71.
II. Mikrobiologisches über die Arrakfabrikation in Batavia.—C. f. B. 1. Abt. 1894. XVI. 97.—Ch. C. 1894. II. 614. —K. J. V. 149.

EISENBERG, James,
I. Bakteriologische Diagnostik. 3rd Ed. 1891. Hamburg. Voss.

EISENSCHITZ, Siddy,
I. Beiträge zur Morphologie der Sprosspilze. Berner Dissert. Wien. 1895.—Z. g. Br. 1895. XVIII. 379.
II. Über die Granulierung der Hefezellen. —C. f. B. 2. Abt. 1895. I. 674.

EITNER, W.,
I. Der Gerber. 1898. XXIV. 4.

EKENSTEIN, van,
I. C. R. 1897. CXXV. 719
II. Recueil des travaux chim. des Pays-Bas. 1896. XV. 221.

ELFVING, Fredrik,
I. Studien über die Einwirkung des Lichtes auf die Pilze.—Helsingfors. 1890.
II. Über Saccharomyces glutinis (Fres.) Cohn.—Öfversigt af Finska Vet. Soc. Förhandl. 1886. XXVIII.

ELION, H.,
I. Studien über Hefe.—C. f. B. 1893. XIV. 53.—K. J. IV. 142.
II. Die Bestimmung von Maltose, Dextrose und Dextrin in Bierwürze und Bier mittelst Reinkulturen von Gärungsorganismen—C. f. B. 1891. IX. 525.—Ch. C. 1891. II. 281.—K. J. II. 141.
III. Züchtung von Ascosporen auf Tonwürfeln.—C. f. B. 1. Abt. 1893. XIII. 749.—K. J. IV. 39.
IV. Reinhefe in der Brauerei.—Z. g. Br. 1888. XI. 33.—C. f. B. 1. Abt. 1892. XI. 192.—K. J. II. 149.
V. Zeitschr. f. angew. Chemie. 1890. 321, 322.

ELLIESEN, Max,
I. Einfluss des Vegetations-zustandes verschiedener Hefen auf ihr Vermehrungs und Gärvermögen.—C. f. B. 2 Abt. 1901. VII. 497.

ELSNER, Fritz,
I. Untersuchungen von Lebensmitteln und Gebrauchsgegenständen. Berlin. 1878. —Zeitschrift gegen Verfälschung der Lebensmittel. 1878. No. 3.—Z. g. Br. 1878. 217, 219.

ELWART,
I. Bull. de l'assoc. des Chimistes. 1901. XX. 562.

EMMERICH, Rudolf,
I. Untersuchungen über die Pilze der Cholera asiatica.—A. f. Hygiene. 1885. III. 291.

EMMERLING, O.,
I. Zur Frage, wodurch die Giftigkeit arsenhaltiger Tapeten bewirkt wird.—Ber. d. D. Chem. Ges. 1896. XXIX. 2728. 1897. XXX. 1026.—Ch. C. 1897. I. 119, 1218.—K. J. VII. 231.
II. Über armenisches Mazun.—C. f. B. 2. Abt. 1898. IV. 418.—Ch. C. 1898. II. 441.
III. Zur Kenntnis des Sorbosebakteriums. —Ber. d. D. Chem. Ges. 1899. XXXII. 541.—C. f. B. 2. Abt. 1899. V. 657.—Ch. C. 1899. I. 853.
IV. Über Schimmelpilzgärung.—Ber. d. D. Chem. Ges. 1897. XXX. 454.—Ch. C. 1897. I. 767.—C. f. B. 2. Abt. 1897. III. 322.—K. J. VIII. 107.
V. Ber. d. D. Chem. Ges. 1902. XXXV. 2289.
VI. C. f. B. 2. Abt. 1903. X. 273.
VII. Ber. d. D. Chem. Ges. 1902. XXXV. 694.
VIII. *Ibid.* 1899. XXXII. 544.
IX. *Ibid.* 1896. XXIX. 2726.
X. *Ibid.* 1897. XXX. 451.
XI. Z. f. Spiritusindustrie. 1904. XXVII. 477.
XII. Ber. d. D. Chem. Ges. 1901. XXXIV. 3810.
XIII. *Ibid.* XXXIV. 600.

ENGEL,
I. Les ferments alcooliques. Thèse. Paris. 1872.
II. *Ibid.* Thèse. Paris. 1872. 52.

ENGELKE,
I. Hedwigia. 1902. XLI. Dec.

ENGELMANN, Th. W.,
I. Bacterium photometricum. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes. — Pflüger's Archiv. 1883. XXX. 95. With plate.

II. Neue Methode zur Untersuchung der Sauerstoff-Ausscheidung pflanzlicher und tierischer Organismen.—Bot. Ztg. 1881. XXXIX. 441.—Ch. C. 1881. 561.
III. Über Sauerstoff-Ausscheidung von Pflanzenzellen im Mikrospectrum.—Bot. Ztg. 1882. XI. 419, 321.
IV. Die Erscheinungsweise der Sauerstoff-Ausscheidung chromophyllhaltiger Zellen im Licht bei Anwendung der Bakterienmethode. With plate. Pflüger's Archiv. 1894. LVII. 375.—Ch. C. 1894. II. 481.—K. J. V. 27.
V. Die Purpurbakterien und ihre Beziehungen zum Licht.—Bot. Ztg. 1888. XXXXVI. 661.—C. f. B. 1889. V. 569.—Ch. C. 1889. I. 80.
VI. Über Bacteriopurpurin und seine physiologische Bedeutung.—Pflüger's Archiv. 1888. XXXXII. 183.—Ch. C. 1888. 581.
VII. Zur Biologie der Schizomyceten.—Bot. Ztg. 1882. XL. 321.

Engler, Ad.,
I. Über die Pilzvegetation des weissen oder toten Grundes der Kieler Bucht.—Bericht d. Kommission z. Erforschung der deutschen Meere. 1881.—Ber. d. Botan. Ver. d. Prov. Brandenburg. 1882. 19.

Engler, A., u. Prantl, K.,
I. Die natürl. Pflanzenfamilien. 1900. Lief. 196 and 197, 454.

Epstein,
I. Arch. f. Hygiene. 1902. XLV. 354.

Erdmann, O.,
I. Bildung von Anilinfarben aus Proteinkörpern.—Journal f. prakt. Chemie. 1866. LXXXXIX. 385.

Eriksson, Jakob,
I. Studier öfver leguminosernas rotknölar.—Acta Univ. Lund. 1873. X.; bez. Akademisk Afhandlingar. Lund. 1874.

Erlenmeyer u. Planta, A. v.,
I. Biedermann's Centralbl. 1875. VII. 25.

Ermann,
I. Beitrag z. Kenntnis der Fettwachsbildung.—Vierteljahrsschrift für gerichtl. Medicin. 1882.

Ermengem, E. van,
I. Sterilisation der Wässer durch Ozon.—Ann. Inst. Past. 1895. IX. 673.—Ch. C. 1895. II. 999.

Ermengem, E. van, und Sugg,
I. Über die desinfizierende Wirkung des Formalins.—C. f. B. 1. Abt. 1896. XIX. 91.—Ch. C. 1896. I. 934.—K. J. VII. 72.

Ernst, Paul,
I. Über den Bacillus xerosis und seine Sporenbildung.—Z. f. Hygiene. 1888. IV. 25.—C. f. B. 1888. II. 47.
II. Über Kern- und Sporenbildung in Bakterien.—Z. f. Hygiene. 1889. V. 428.—C. f. B. 1889. V. 796.
III. Über einen neuen Bacillus des blauen Eiters.—Z. f. Hygiene. 1887. II. 369.—C. f. B. 1887. II. 276.—Ch. C. 1887. 1168.

Errera, Léo,
I. Expériences relatives à l'action des rayons X sur un Phycomyces.—C. R. 1896. CXXII. 787.
II. L'épiplasme des Ascomycètes et le glycogène des végétaux.—Thèse d'agrégation. Bruxelles. 1882.
III. Sur le glycogène chez les Mucorinées.—Bulletin de l'Acad. Roy. de Belg. 1882. 3e sér. IV. 451.
IV. Sur le glycogène chez les Basidiomycètes.—Mémoires de l'Ac. Roy. de Belgique; Collection in 8°. 1885. XXXVII.—Bot. Ztg. 1886. XXXXIV. 200, 316.
V. Sur l'existence du glycogène dans la levure de bière.—C. R. 1885. CI. 253.—Z. g. Br. 1885. VIII. 333.—Ch. C. 1885. 685.
VI. Les réserves hydrocarbonnées des Champignons.—C. R. 1885. CI. 391.—Z. g. Br. 1885. VIII. 369.
VII. Anhäufung und Verbrauch von Glycogen bei Pilzen.—Ber. d. D. Bot. Ges. 1887. V. 500.—Z. g. Br. 1888. XI. 37.
VIII. Die grosse Wachstumsperiode bei den Fruchtträgern von Phycomyces.—Bot. Ztg. 1884. XXXXII. 497.
IX. On the Cause of Physiological Action at a Distance.—Annals of Botany. 1892. VI. No. 24.

Erxleben,
I. Die Güte und Stärke des Bieres. Prag. 1818. 69.

Esaulow, Nikolai,
I. Über Kefir, eine bakteriolog. u. chem. Untersuchung. Dissert. Moskau. 1895. In Russian.—Pharmac. Zeitschrift f. Russland. 1895. XXXIV. 232.—Ch. C. 1895. I. 1072.—K. J. VI. 222.

Eschenhagen, Franz,
I. Über den Einfluss von Lösungen verschiedener Konzentration auf das Wachstum von Schimmelpilzen. Ein Beitrag zur Kenntnis der Rolle, welche der Turgor in niederen Organismen spielt.—Leipziger Dissert. 1889. Stolp.—Ch. C. 1890. II. 250.

Escherich, Theodor,
I. Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung. Stuttgart. 1886.—Fortschritte der Medicin. 1885. No. 16 and 17.—Baumgarten's Jahresbericht. 1885. I. 169.

Escombe, F.,
I. Beitrag zur Chemie der Membranen der Flechten und Pilze.—Z. f. physiolog. Chemie. 1896. XXII. 288.—C. f. B. 2. Abt. 1897. III. 195.—Ch. C. 1897. I. 89.

ESMARCH, Erwin von,
I. Über die Reinkultur eines Spirillum.—C. f. B. 1887. I. 225.
II. Der Henneberg'sche Desinfektor.—Z. f. Hygiene. 1887. II. 342.—C. f. B. 1887. II. 234.
III. Über eine Modifikation des Koch'schen Plattenverfahrens zur Isolierung und zum quantitativen Nachweis von Mikroorganismen.—Z. f. Hygiene. 1886. I. 293.—Ch. C. 1886. 699.

ETARD, A., et OLIVIER, L.,
I. De la réduction des sulfates par les êtres vivants.—C. R. 1882. XCV. 846.

EUGLING, Wilhelm,
I. Studien über das Kasein der Kuhmilch und über die Labfermentwirkung. —D. Landw. Versuchsstationen. 1885. XXXI. 391.

EULER, H.,
I. Z. f. physiolog. Chemie. 1905. XLIV. 53.

EVANS,
I. Die Beseitigung der Salpetersäure im Brauwasser und deren Einfluss auf die Hefe.—Journal of the Federated Institutes of Brewing. 1896. II. 195.—W. f. Br. 1896. XIII. 521.—Ch. C. 1896. II. 218.—K. J. VII. 92.

EWART, J. Cossar,
I. The Life-History of Bacterium termo. —Proceedings of the Royal Society of London. 1878, p. 474.

FABIAN, A.,
See NENCKI und FABIAN.

FABRIZIO ab Aquapendente, Geronimo,
I. De visione, voce et auditu. Venetia. 1600.

FAGET,
I. Liebig's Ann. 1853. LXXXVIII. 325.
II. *Ibid.* 1862. CXXIV. 355.

FALKE, Friedrich,
I. Die Braunheu-Bereitung.—Arbeiten der D. Landwirtschafts-Gesellschaft. 1895. Heft 9.

FAMINTZIN, A.,
I. Eine neue Bakterienform, Nevskia ramosa.—Bulletin de l'Acad. de St. Pétersbourg. Nouvelle série (II). 1891. XXXIV. 481.—Ch. C. 1892. I. 636.—K. J. II. 42.

FEDOROLF, A. K.,
I. Der Einfluss des Chlorlithiums auf Bakterien.—Wratsch 1895. XVI. 1084.—Ch. C. 1896. I. 266.

FEHLING,
I. Dingler's Journ. 1853. CXXX. 77.

FEILITZEN, Hjalmar von, und TOLLENS, B.,
I. Gärungsversuche mit Torf.—Journal f. Landwirtschaft. 1898. XXXXVI. 23.—Ber. d. D. Chem. Ges. 1897. XXX. 2577.—Ch. C. 1898. I. 35.—K. J. VIII. 131.

FELTZ, E.,
I. Über das Auftreten gallertartiger Stoffe bei den Walzenpressen.—Sucrerie indigène. 1874. No. 10. 1876. No. 14.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1875. 109. 1876. 749. 1877. 464.

FERMI, Claudio,
I. Über die Reinigung der Abwässer durch Elektrizität.—A. f. Hygiene. 1891. XIII. 206.—K. J. II. 95.
II. Die Leim und Fibrin lösenden und die diastatischen Fermente der Mikroorganismen.—A. f. Hygiene. 1890. X. 1.—C. f. B. 1890. VII. 469.—K. J. I. 159.
III. Weitere Untersuchungen über die tryptischen Enzyme der Mikroorganismen.—C. f. B. 1891. X. 401.—K. J. II. 254.
IV. Die Leimgelatine als Reagens zum Nachweis tryptischer Enzyme.—A. f. Hygiene. 1891. XII. 240.—K. J. II. 252.

FERMI, Cl., und BUSCAGLIONI,
I. Die proteolytischen Enzyme im Pflanzenreiche.—C. f. B. 2. Abt. 1899. V. 24.—Ch. C. 1899. I. 501.

FERMI, Cl., und MONTESANO, G.,
I. Die von den Mikroben bedingte Inversion des Rohrzuckers.—C. f. B. 2. Abt. 1895. I. 482.—Ch. C. 1895. II. 712.

FERMI, Cl., und PERNOSSI, L.,
I. Über die Enzyme.—Z. f. Hygiene. 1894. XVIII. 83.—C. f. B. 1894. XV. 229; XVI. 830.—Ch. C. 1894. I. 965.—K. J. V. 280.

FERMI, Cl., u. POMPONI, E.,
I. C. f. B. 2. Abt. 1896. II. 574.

FERNBACH, A.,
I. De l'absence des microbes dans les tissus végétaux.—Ann. Inst. Past. 1888. II. 567.—C. f. B. 1888. IV. 713.
II. Der Amylomyces Rouxii und seine Verwendung in der Brennerei.—Z. f. Spiritusind. 1899. XXI. Ergänzungsheft. I. 57.
III. C. R. 1900. CXXXI. 1214.
IV. Annales de la brasserie et distillerie. 1899. II. 409.
V. Recherches sur la sucrase. Paris. 1890.
VI. Ann. Pasteur. 1890. IV. 641.
VII. *Ibid.* 1889. III. 473, 531.
VIII. *Ibid.* 1890. IV. 1.

FERNBACH, A., et HUBERT, L.,
I. Sur la diastase protéolytique du malt.—C. R. 1900. CXXXI. 1783.—Ch. C. 1900. II. 391.

FERRIER,
I. Considérations générales sur le pléomorphisme des cils vibratiles de quelques bactéries mobiles.—Archives de médecine expérimentale et d'anatomie pathologique. Série I. T. VII.—C. f. B. 2. Abt. 1895. I. 497.

Fesca, Max, und Imai,
I. Über die Bestandteile des Tabakes und über die für die Beurteilung und Untersuchung desselben wichtigsten Momente. — Landw. Jahrbücher. 1888. XVII. 329.—Ch. C. 1888. 1121.

Fick, A.,
I. Zu P. Walther's Abhandlung über Fick's Theorie der Labwirkung, etc.— Pflüger's Archiv. 1891. XXXXIX. 110.—K. J. II. 258.

Fiechter,
I. Wirkung d. Blausäure auf Fermente. Dissert. Basel. 1875.

Fisch, C.,
I. Über die Pilzgattung Ascomyces.—Bot. Ztg. 1885. XXXXIII. 33.

Fischel, F.,
I. Untersuchungen über die Morphologie und Biologie des Tuberkulose-Erregers. —Fortschritte d. Medizin. 1892. X. No. 22.—C. f. B. 1893. XIII. 134.

Fischer, A.,
I. Liebig's Ann. 1860. CXV. 247. 1861. CXVIII. 307.

Fischer, Alfred,
I. Die Plasmolyse der Bakterien.—Ber. d. K. sächs. Ges. d. Wissenschaften. Mathem.-phys. Klasse. Leipzig. 1891.— K. J. II. 63.
II. Untersuchungen über Bakterien.— Jahrbücher für wissenschaftl. Botanik. 1894. XXVII. 163.—C. f. B. 2. Abt. 1895. I. 701.—K. J. V. 45.
III. Die Pilze Deutschlands, Osterreichs und der Schweiz. IV. Abt.: Phycomycetes. Leipzig. 1892. Ed. Kummer.

Fischer, B., u. Brebeck, K.,
I. Zur Morphologie, Biologie, u. Systematik d. Kahmpilze, d. Monilia candida Hansen u. d. Soorpilzes. Jena. 1894.

Fischer, Bernhard,
I. Bakterienwachstum bei 0°, sowie über das Photographieren von Kulturen leuchtender Bakterien in ihrem eigenen Lichte.—C. f. B. 1888. IV. 89.
II. Über einen neuen lichtentwickelnden Bacillus.—C. f. B. 1888. III. 105.

Fischer, Bernhard, und Proskauer, B.,
I. Über die Desinfektion mit Chlor und Brom.—Mitteil. a. d. K. Gesundh.-Amte. 1884. II. 228.—Ch. C. 1884. 222.

Fischer, Ed.,
I. Die Pilze Deutschlands, Österreichs und der Schweiz. V. Abt.: Tuberaceen und Hemiasceen. Leipzig. 1897.
II. In Engler-Prantl. Nat. Pflanzenfamilien. Teil I. 1900. 537.

Fischer, Emil,
I. Synthesen in der Puringruppe.—Ber. d. D. Chem. Ges. 1899. XXXII. 435.— Ch. C. 1899. I. 834.
II. Ber. d. D. Chem. Ges. 1890. XXIII. 270.
III. *Ibid.* 1895. XXVIII. 1432.
IV. *Ibid.* 1894. XXVII. 3481.
V. *Ibid.* 1891. XXIV. 1836.
VI. W. f. Br. 1897. XIV. 363.
VII. Ber. d. D. Chem. Ges. 1894. XXVII. 2988, 3251.
VIII. *Ibid.* 1895. XXVIII. 3037.
IX. Verhandl. d. Ges. Naturforscher u. Aerzte. Wien. 1895. Bericht. 109.
X. Ber. d. D. Chem. Ges. 1887 XX. 1089, 3384. 1888. XXI. 2634. 1889. XXII. 97, 106.
XI. *Ibid.* 1890. XXIII. 2226.
XII. *Ibid.* 1895. XXVIII. 1429.
XIII. *Ibid.* 1890. XXIII. 3687.
XIV. *Ibid.* 1895. XXVIII. 3024.
XV. *Ibid.* 1894. XXVII. 2985.
XVI. *Ibid.* 1894. XXVII. 2031.
XVII. *Ibid.* 1889. XXII. 3218.

Fischer, Emil, u. Armstrong,
I. Ber. d. D. Chem. Ges. 1902. XXXV. 3151.

Fischer, Emil, und Lindner, Paul,
I. Über die Enzyme einiger Hefen.—W. f. Br. 1895. XII. 959.—C. f. B. 2. Abt. 1895. I. 889.—Ch. C. 1895. II. 1048.
II. *Ibid.* II.—Ber. d. D. Chem. Ges. 1895. XXVIII. 3034.—C. f. B. 2. Abt. 1895. I. 889.—Ch. C. 1896. I. 265.— K. J. VI. 323.
III. Ber. d. D. Chem. Ges. 1895. XXVIII. 3037.
IV. *Ibid.* 1895. XXVIII. 984.

Fischer, E., u. Niebel,
I. Sitz. d. Preuss. Akad. d. Wissen. 1896. Jan. 30.

Fischer, Emil, und Roeder, G.,
I. Synthese des Thymins und anderer Uracile.—Sitzgsber. d. Akad. d. Wiss. Berlin. 1901. XII. 268.—Ch. C. 1901. I. 887.

Fischer, Emil, und Thierfelder, H.,
I. Verhalten der ! verschiedenen Zucker gegen reine Hefen.—Ber. d. D. Chem. Ges. 1894. XXVII. 2031.—C. f. B. 2. Abt. 1895. I. 121.—Ch. C. 1894. II. 360.—K. J. V. 145.

Fischer, H.,
I. C. f. B. 2. Abt. 1902. IX. 353, 385.
II. *Ibid.* 1903. X. 452.

Fischer von Waldheim, Alexander,
I. Über Heliotropismus bei niederen Pilzen und speziell bei Pilobolus.—Arbeiten des bot. Laboratoriums d. Kais. Universität Warschau. 1875. Heft I.— Bot. Ztg. 1876. XXXIV. 479.

Fittig,
I. Zeits. f. Chemie. 1867. II. 44.

Fitz, Albert,
I. Über Schizomyceten-Gärungen.—Ber. d. D. Chem. Ges. 1876. IX. 1348.
II. *Ibid.* II.—*Ibid.* 1877. X. 276.
III. *Ibid.* III.—*Ibid.* 1878. XI. 42.
IV. Über Spaltpilzgärungen. IV. *Ibid.* 1878. XI. 1890. — Dingl. Journal. 1879. CCXXXI. 191.
V. *Ibid.* V.—*Ibid.* 1879. XII. 474.
VI. *Ibid.* VI.—*Ibid.* 1880. XIII. 1309.

VII. *Ibid.* VII.—*Ibid.* 1882. XV. 867.
VIII. Notizen über die Gärung.—Annalen der Önologie. 1870. I. 437.
IX. Über alkoholische Gärung durch Mucor mucedo.—Ber. d. D. Chem. Ges. 1873. VI. 48.
X. Über alkoholische Gärung durch den Schimmelpilz Mucor racemosus.—Ber. d. D. Chem. Ges. 1875. VIII. 1540.
XI. Über alkoholische Gärung.—Ber. d. D. Chem. Ges. 1876. IX. 1352.
XII. Ann. d. Önologie. 1872. II. 428.
XIII. Ber. d. D. Chem. Ges. 1877. X. 278.
XIV. *Ibid.* 1880. XIII. 36.

Fleck, H.,
I. Quoted by Will, I.
II. Ber. d. Chem. Centralstelle. Dresden. 1876.

Fleischer, M.,
I. Über Bodenimpfung, ihre Ergebnisse und ihre Aussichten.—Chemikerzeitg. 1893. XVII. 900.—K. J. IV. 222.

Fleischmann, W.,
I. Lehrbuch der Milchwirtschaft. 1893. Bremen. M. Heinsius Nachf.

Flemming,
I. Zellsubstanz, Kern und Zellteilung. Leipzig. 1882.

Flückiger, F. A.,
I. Pharmakognosie des Pflanzenreiches. 1891. 3rd Ed. Berlin. R. Gaertner.

Flügge, C.,
I. Die Mikroorganismen. 3rd Ed. 1896. Zwei Bände. Leipzig. Vogel.
II. Die Aufgaben und Leistungen der Milchsterilisierung gegenüber den Darmkrankheiten der Säuglinge.—Z. f. Hygiene. 1894. XVII. 272.—Ch. C. 1894. II. 385.—K. J. V. 226.

Flügge, C., und Sirotinin,
I. Studien über die Abschwächung virulenter Bakterien und die erworbene Immunität.—Z. f. Hygiene. 1888. IV. 208, 262.—C. f. B. IV. 593, 636.

Flühler, Adalbert,
I. Die Säurebildung beim Mälzen und Brauen.—Der bayr. Bierbrauer. 1872. VII. 167. 1873. VIII. 24.

Fodor, J. von,
I. Bakterien im Blute lebender Tiere.—A. f. Hygiene. 1886. IV. 129.—Ch. C. 1886. 922.

Fokker, A. P.,
I. Über das Milchsäureferment.—Fortschritte der Medizin. 1889. No. 11.—C. f. B. 1889. VI. 293.—K. J. I. 83.
II. Onderzoekingen over melkzuur gisting.—Weekblad van het Ned. Tijdschrift voor Geneeskunde. 1890. 88, 509.—C. f. B. 1890. VIII. 426.—K. J. I. 83.

Fonseca, Ant., und Chiaromonte, Tom.
I. Der Zusatz von Säuren zum Most und Wein.—Staz. sperim. agrar. ital. 1894. XXV. 20.—Ch. C. 1894. I. 246.

Fonssagrives,
I. Sur l'Oïdium aurantiacum.—C. R. 1871. LXXIII. 781.

Fordos,
I. Recherches sur la matière colorante des suppurations bleues: pyocyanine.—C. R. 1860. LI. 215.—Journal f. prakt. Chemie. 1862. LXXXV. 249.

Forster, J.,
I. Über einige Eigenschaften leuchtender Bakterien.—C. f. B. 1887. II. 337.
II. Über die Entwicklung von Bakterien bei niederen Temperaturen.—C. f. B. 1892. XII. 431.—K. J. III. 63.
III. Über den Einfluss des Räucherns auf die Infektiosität des Fleisches perlsüchtiger Rinder.—Münchener medizinische Wochenschrift. 1890. No. 16.—C. f. B. 1890. VIII. 79.

Forster, J., und Man, C. de,
I. Über die Einwirkung hoher Temperaturen auf Tuberkel-Bacillen.—Hygien. Rundschau. 1892. II. 869. 1893. III. 669.—Archiv. f. Hygiene. 1893. XVIII. 134.—Ch. C. 1893. II. 941.—C. f. B. 1893. XIII. 299.

Förster, K.,
I. Über den Furfurolgehalt gegorener Flüssigkeiten.—Ber. d. D. Chem. Ges. 1882. XV. 322.

Forti, Cesare,
I. Relazione sugli studi zimotecnici. I. e II.—Bolletino di Notizie agrarie. 1896. XVIII. 363.—C. f. B. 2. Abt. 1897. III. 122.—K. J. VII. 131.

Foth, G.,
I. W. f. Br. 1887. IV. 73, 305.
II. *Ibid.* 1889. VI. 263.

Foureur, A.,
See Wurtz et Foureur.

Foutin, W. M.,
I. Bakteriologische Untersuchungen von Hagel.—Wratsch. 1889. No. 49 u. 50.—C. f. B. 1890. VII. 372.

Fox, Joseph. J.,
See Frankland and Fox.

Fraenkel, Carl,
I. Die Einwirkung der Kohlensäure auf die Lebenstätigkeit der Mikroorganismen.—Zeitschrift f. Hygiene. 1888. V. 332. Gives antecedent literature.—C. f. B. 1889. V. 208.—Ch. C. 1889. I. 49.
II. Beiträge zur Kenntnis des Bakterien wachstums auf eiweissfreien Nährböden.—Hygien. Rundschau. 1894. IV. 769.—C. f. B. 2. Abt. 1895. I. 252.—K. J. V. 13.
III. Untersuchungen über das Vorkommen von Mikroorganismen in verschiedenen bodenschichten.—Z. f. Hygiene. 1887. II. 521.—C. f. B. 1888. III. 235.—Ch. C. 1887. 1170.
IV. Über die Kultur anaerober Mikroorganismen.—C. f. B. 1888. III. 735.—Ch. C. 1888, 1036.

V. Über den Bakteriengehalt des Eises. —Z. f. Hygiene. 1886. I. 302.—Ann. Inst. Past. 1887. I. 136.

FRAENKEL, C., u. PFEIFFER, R.,

I. Mikrophotographischer Atlas der Bakterienkunde. 2nd Ed. 1893. Berlin. A. Hirschwald. 60*s.*

FRAENKEL, S.,

See KERRY und FRAENKEL.

FRANCKE, G.,

I. Über Bakterientrübung im Bier.—W. f. Br. 1884. I. 727.

FRANK, Albert Bernhard,

I. Mitteilung betreffs in einem Rohzucker-Nachprodukt vorgefundener, gefärbter Pilze. — Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. D. Reiches. 1891. 662.—C. f. B. 1892. XII. 661. —K. J. II. 225.

II. Die Krankheiten der Pflanzen. 2nd Ed. I. Bd.: Die durch anorgan. Einflüsse hervorgerufenen Krankheiten. 6*s.*—II. Bd.: Die Pilzkrankheiten der Pflanzen. 10*s.* 10*d.*—Breslau. Trewendt.

III. Über die Parasiten in den Wurzelanschwellungen der Papilionaceen. — Bot. Ztg. 1879. XXXVII. 832.

IV. Die Assimilation des freien Stickstoffes bei den Pflanzen. — Landw. Jahrb. 1892. XXI. 1.—Bot. Ztg. 1. Abt. 1893. LI. 139.—Ch. C. 1894. I. 172.—K. J. III. 194; IV. 224.

V. Sind die Wurzelanschwellungen der Erlen und Eleagnaceen Pilzgallen? — Ber. d. D. Bot. Ges. 1887. V. 50. —C. f. B. 1887. I. 606.

VI. Über die Pilzsymbiose der Leguminosen.—Ber. d. D. Bot. Ges. 1889. VII. 332.—Landw. Jahrbücher. 1890. XIX. 523.—C. f. B. 1890. VII. 413. 1891. IX. 629.—K. J. I. 120.

VII. Über den Dimorphismus der Wurzelknöllchen der Erbse.—Ber. d. D. Bot. Ges. 1892. X. 170.—C. f. B. 1892. XII. 271.—K. J. III. 200, 203.

VIII. Über die auf den Gasaustausch bezüglichen Einrichtungen und Tätigkeiten der Wurzelknöllchen der Leguminosen.—Ber. d. D. Bot. Ges. 1892. X. 271.—K. J. III. 198.

IX. Über die Ursache der Nitrifikation der Ammoniaksalze im Erdboden.—Deutsche landw. Presse. 1887. XIV. No. 104.

FRANKLAND, Percy F.,

I. Über den Einfluss der Kohlensäure und anderer Gase auf die Entwicklungsfähigkeit der Mikroorganismen.— Z. f. Hygiene. 1889. VI. 13.—C. f. B. 1889. VI. 261.

II. Die Wirkung einiger Mikroorganismen auf Salpetersäure. — Chemical News. 1888. LVII. 89.—Ch. C. 1888. 553.

III. The Nitrifying Process and its Specific Ferment. — Transactions of the Royal Society of London. 1890. CLXXXI. 107.—K. J. I. 107.

IV. Trans. Chem. Soc. 1891. — Chem. News. 1891. LXIII. 136.

V. Journ. Chem. Soc. 1891. LIX. 253. 1892. LX. 432, 737.

VI. *Ibid.* 1892. LX. 254.

FRANKLAND, P. F., and FOX, J. J.,

I. On a Pure Fermentation of Mannite and Glycerin. — Proceedings of the Royal Society. 1889. XXXXVI. 345.—C. f. B. 1890. VII. 241.—Ch. C. 1889. II. 1027.

FRANKLAND, P. F., and FREW, W.,

I. The Fermentation of Calcium Glycerate by the Bacillus ethaceticus.—Journal of the Chemical Society. 1891. LIX.-LX. 81.—C. f. B. 1892. XII. 724.—K. J. II. 237.

II. A Pure Fermentation of Mannitol and Dulcitol.—Journal of the Chemical Society. Transactions. 1892, p. 254. —C. f. B. 1892. XII. 252. — K. J. III. 229.

III. An Optically Active Glyceric Acid. — Journal of the Chemical Society. 1891. LIX.-LX. 96.—Ch. C. 1891. I. 528.—K. J. II. 238.

FRANKLAND, P. F., and LUMSDEN, J. S.,

I. Decomposition of Mannitol and Dextrose by the Bacillus ethaceticus. — Journal of the Chemical Society. Transactions. 1892, p. 432.—Ch. C. 1893. I. 46.—K. J. III. 231.

FRANKLAND, P. F., and MACGREGOR, J.,

I. Fermentation of Arabinose with the Bacillus ethaceticus.—Journal of the Chemical Society. Transactions. 1892, p. 737. — C. f. B. 1892. XII. 725. —Ch. C. 1892. II. 532.—K. J. III. 232.

FRANKLAND, P. F., STANLEY, A., and FREW, W.,

I. Fermentations induced by the Pneumococcus of Friedländer. Transactions of the Chemical Society. 1891.—Chemical News. 1891. LXIII. 136.—C. f. B. 1891. X. 222.—Ch. C. 1891. I. 704. 1892. I. 217.—K. J. II. 234.

FRANKLAND, P. F., and WARD, M. H.,

I. First Report to the Water Research Committee of the Royal Society on the Present State of our Knowledge concerning the Bacteriology of Water, with Especial Reference to the Vitality of Pathogenic Schizomycetes in Water.— Proceedings of the Royal Society. 1892. LI. 183.

II. Second Report: The Vitality and Virulence of Bacillus anthracis and its Spores in Potable Waters.—Proceedings, etc. 1893. LIII. 164.

FREMLIN,

I. Vergleichende Studien an Bacterium coli commune verschiedener Provenienz. —A. f. Hygiene. 1893. XIX. 295.—C. f. B. 1894. XV. 693.—Ch. C. 1894. I. 162.—K. J. IV. 87.

FRÉMY, Edmond,
I. Recherches sur la composition chimique des tissus des végétaux. — Journal de Pharmacie et de chimie. 1859. XXXVI. 5.—Compare KABSCH (I.).

FRÉMY, Edmond, et BOUTRON-CHALARD, Ant. Franç.,
I. Sur la fermentation lactique.—C. R. 1841. XII. 728.—Ann. de Chim. et de Phys. (3.) 1841. II. 257.—Journal f. prakt. Chemie. 1841. XXIV. 51, 364.

FRENZEL, J.,
I. Über den Bau und die Sporenbildung grüner Kaulquappen-Bacillen. — Z. f. Hygiene. 1891. XI. Heft 2.—K. J. II. 47.

FRESENIUS,
I. Beiträge z. Mykologie. Frankfurt. 1850–1863.
II. *Ibid.* Frankfurt. 1853. I. 22.
III. Z. f. analyt. Chem. 1891. XXX. 669.

FREUDENREICH, Eduard von,
I. Über den jetzigen Stand der bakteriologischen Forschung auf dem Gebiete des Käsereifungs-processes.—C. f. B. 2. Abt. 1895. I. 854.—Ch. C. 1896. I. 451.
II. De la teneur du lait en bactéries.—Annales de micrographie. 1890. II. 115. —K. J. I. 82.
III. Bakteriologische Untersuchungen über den Reifungsprocess des Emmenthaler Käses. — Landw. Jahrbuch d. Schweiz. 1891. V. 16.—C. f. B. 1892. XII. 334.—K. J. II. 195.
IV. Weitere bakteriologische Untersuchungen über den Reifungsprocess des Emmenthaler Käses.—Landw. Jahrbuch d. Schweiz. 1894. VIII. 207.—C. f. B. 2. Abt. 1895. I. 168.—K. J. V. 240.
V. Recherches préliminaires sur le rôle des bactéries dans la maturation du fromage d'Emmenthal. — Annales de micrographie. 1890. II. 257.—K. J. I. 92.
VI. Sur quelques bactéries produisant le boursouflement des fromages.—Annales de micrographie. 1890. II. 553.—C. f. B. 1890. VIII. 300.—K. J. I. 95.
VII. Über einen neuen, in geblähtem Käse gefundenen Bacillus (B. Schafferi).—Landw. Jahrbuch d. Schweiz. 1890. IV. 17.—K. J. I. 96.
VIII. Über den Einfluss der bei dem Nachwärmen des Käses angewandten Temperatur auf die Bakterienzahl in der Milch und im Käse.—Landw. Jahrbuch d. Schweiz. 1895. IX. 100.—C. f. B. 2. Abt. 1895. I. 760.—K. J. VI. 246.
IX. Über einige Versuche die Blähung der Käse zu verhindern.—Landw. Jahrbuch d. Schweiz. 1893. VII. 81.—K. J. IV. 206.
X. Beitrag zur Kenntnis der Ursachen des bittern Käses und der bittern Milch. —Landw. Jahrbuch d. Schweiz. 1894. VIII. 135.—C. f. B. 2. Abt. 1895. I. 507.—K. J. V. 222.
XI. Bakteriologische Untersuchungen über den Kefir. —Landw. Jahrbuch d. Schweiz. 1896. X. 1.—C. f. B. 2. Abt. 1897. III. 47.—Ch. C. 1897. I. 872.—K. J. VII. 162.

FREUDENREICH, Ed. von, u. JENSEN, Orla,
I. Über den Einfluss des Naturlabes auf die Reifung des Emmenthaler Käses. —C. f. B. 2. Abt. 1897. III. 545.—Ch. C. 1898. I. 73.
II. C. f. B. 2. Abt. 1897. III. 552.

FREUDENREICH, Ed. v., u. SCHAFFER, F.,
I. Über den Einfluss des Luftabschlusses auf die Reifung des Emmenthaler Käses. — Landw. Jahrbuch d. Schweiz. 1892. VI.—C. f. B. 1893. XIV. 139. —K. J. III. 186.

FREUND, Aug.,
I. Die Produkte der sauren Gärung der Weizenkleie.—Journal f. prakt. Chemie. (2.) 1871. III. 224.—Ch. C. 1871. 306.

FREW,
I. Journ. Soc. Chem. Industry. 1898. XVII. 561.

FREW, William,
See FRANKLAND and FREW, *and* FRANKLAND, STANLEY, and FREW.

FREY, H.,
I. Über die Zersetzungsprodukte der im menschlichen Dünndarm vorkommenden Mikroben.—Schweiz. Wochenschrift f. Pharmacie. 1891. XXIX. 111. — Ch. C. 1891. I. 833.—K. J. II. 240.

DE FREYTAG, C. J.,
I. Über die Einwirkung konzentrierter Kochsalzlösungen auf das Leben von Bakterien.—A. f. Hygiene. 1890. XI. 60.—Ch. C. 1890. II. 449.

FRIEDEL,
I. Bull. Soc. Bot. de France. 1905. LII. 182.
II. *Ibid.* 1904. LI. 209.—C. R. 1904. CXXXVIII. 1118.

FRIEDENTHAL, H.,
I. Über den Einfluss von Induktions-Elektrizität auf Bakterien.—C. f. B. 1. Abt. 1896. XX. 505.—Ch. C. 1896. II. 939.—K. J. VII. 54.

FRIEDLÄNDER, P.,
I. Der Mikrokokkus der Pneumonie.—Fortschritte der Medizin. 1883. I. 715.

FRIES, Elias Magnus,
I. Systema mycologicum. 1821–1829. Greifswald.
II. Systema orbis vegetabilis. 1825. Lund.

FRIIS, F., LUNDE, H. P., u. STORCH, V.,
I. Syrningsforsög. — 32. Beretning fra den Kgl. Veterinär- og Landbohojskoles Laboratorium for Landökonomiske Forsög. Kjöbenhavn. 1895.—C. f. B. 2. Abt. 1895. I. 440.

FRISCH,
I. Über den Einfluss niederer Temperaturen auf die Lebensfähigkeit der Bakterien. —Sitzungsberichte der Wiener Akademie. 1877. 3. Abt. LXXV. 257. 1879. LXXX. 77.

FROMME, Arnold,
I. Über die Beziehungen des metallischen Eisens zu den Bakterien und über den Wert des Eisens zur Wasserreinigung. —Dissert. Marburg. 1891.—C. f. B. 1892. XII. 274.—Ch. C. 1891. II. 180.—K. J. II. 98.

FRY, George,
I. The Theory and Practice of Sweet Ensilage. London. 1885. — German transl.: Die Einsüssung der Futtermittel, Theorie und Praxis der süssen Ensilage nach George Fry. — Berlin. 1885. Parey. 1*s*.—Z. f. Spiritusind. 1885. VIII. 704.

FUCHS, C. J.,
I. Beiträge zur näheren Kenntnis der gesunden und fehlerhaften Milch der Haustiere.—Gurlt und Hertwig's Magazin f. d. gesamte Tierheilkunde. Berlin. 1841. 182.

FUNK, C., und BALOGH, N. von,
I. Verfahren zur Vergärung von Maischen, Teigen, Würzen, etc. German Patent No. 57865.—K. J. II. 170.

FÜISTING, W.,
I. Beiträge zur Entwicklungsgeschichte der Lichenen.—Bot. Ztg. 1868. XXVI. 641, 662.

FÜRBRINGER, P.,
I. Untersuchungen und Vorschriften über die Desinfektion der Hände des Arztes nebst Bemerkungen über den bakteriologischen Charakter des Nagelschmutzes. Wiesbaden. 1888. Bergmann.—C. f. B. 1888. III. 260.

GÄRTNER, A.,
I. Über die Fleischvergiftung in Frankenhausen am Kyffhäuser und den Erreger derselben. — Korrespondenzblatt des allgem. ärztl. Vereins von Thüringen. 1888. No. 9. 573.—Ch. C. 1889. I. 32.

GAILLARD, G.,
I. De l'influence de la lumière sur les Micro-organisms. Lyon. 1888.

GALEAZZI, J.,
I. Ricerche batteriologiche e chimiche sull' incerconimento del vino.—Staz. sper. agr. ital. 1895. XXVIII. 181. —C. f. B. 2. Abt. 1895. I. 892.

GALEOTTI, G.,
I. Ricerche biologiche sopra alcuni bacteri cromogeni. — Lo Sperimentale. 1892. XXXXVI. Fasc. III. p. 261.—C. f. B. 1893. XIV. 696.—K. J. IV. 103.

GALIPPE, M.,
I. Sur la présence de microorganismes dans les tissus végétaux. —La semaine médicale. 1887, p. 267.—C. f. B. 1888. III. 108.—Ch. C. 1888. 554.

GALLI-VALERIO, Bruno, und STRZYZOWSKI, C.,
I. Über den biologischen Arsennachweis. —Pharmac. Post. 1900. XXXIII. 637. —Ch. C. 1901. I. 63.

GANTTER, F.,
I. Über die Durchführung der Nachgärung bei unvollständig vergorenen Weinen.—Bericht ü. d. Verh. d. XV. Deutschen Weinbau-Kongresses in Heilbronn a. N. 1896. 98.

GARNIER,
I. Abst. in Bot. Centralbl. 1904. XCV. 278.
II. Comptes rendus Soc. de Biologie. 1903. Dec. 4 and 8.

GARRÉ, C.,
I. Über Antagonisten unter den Bakterien. —Korrespondenzblatt für Schweizer. Aerzte. 1887. XVII.—C. f. B. 1887. II. 312.—Ch. C. 1887. 1508.

GARROS, F.,
I. Über die Filtration und Sterilisation des Wassers und anderer Flüssigkeiten durch Asbestporcellan. — Le Mercure scientifique. 1892. No. 610, p. 147. —K. J. III. 20.

GASPERINI, G.,
I. Atti Soc. Toscana Sc. Nat. Pisa. 1887. VIII. 326.

GASPERINI, Gustavo,
I. Recherches morphologiques et biologiques sur un microorganisme de l'atmosphère, le Streptothrix Foersteri Cohn.—Annales de micrographie. 1890. II. 449.—K. J. I. 19.
II. Il burro naturale come mezzo di trasmissione della tuberculosi.—Giornale della R. Soc. d'Igiene Milano. 1890.—C. f. B. 1890. VII. 641.

GASTINE, G.,
I. Sur la fermentation alcoolique des miels et la préparation de l'hydromel. —C. R. 1889. CIX. 479. — Ch. C. 1889. II. 956.

GAUTHIER de CLAUBRY,
I. Note sur une altération particulière observée sur le pain.—Annales d'hygiène publique et de médecine légale. 1er sér. 1843. XXIX. 347.—C. R. 1871. LXXIII. 725.
II. De l'altération du pain par diverses espèces de champignons.—Bulletin de l'Académie de médecine. 1871. XXXVI. 729.

GAUTIER, Arm.,
I. Sur une maladie non encore décrite des vins du midi de la France dits vins tournés.—C. R. 1878. LXXXVI. 1338.

GAUTIER, A., et DROUIN, R.,
I. Sur la fixation de l'azote par le sol arable. —C. R. 1891. CXIII. 820.—Ch. C. 1888. 590. 1892. I. 171.—K. J. II. 205.

GAY, Fr.,
I. Die Schwefelwasserstoff-Hefe Crouzel's. —L'union pharmac. 1891. XXXIII. 117.—C. f. B. 1892. XI. 801.—K. J. II. 136.
II. L'Union pharm. 1892. XXXIII. 60.

GAY-LUSSAC, Louis Joseph,
I. Extrait d'un mémoire sur la fermentation. — Annales de Chimie. 1810. LXXVI. 245.
II. Ann. de chim. et de phys. 1815. XCV. 318.
See also PELOUZE et GAY-LUSSAC.

GAYON, H. U.,
I. Etude sur les appareils de pasteurisation des vins.—Extrait de la Revue de viticulture. 1896. Paris.

GAYON, Ulysse,
I. Recherches sur la fermentation du fumier. — C. R. 1884. LXXXVIII. 528.—Ch. C. 1884. 378.
II. Sur les altérations des œufs. — Thèse pour le doctorat. 1875.—C. R. 1877. LXXXV. 1074.
III. Sur l'altération des vins dits "mildiousés."—Revue de viticulture. 1894. I. 33.
IV. Sur la constitution du glucose inactif des sucres bruts de cannes et des mélasses. — C. R. 1878. LXXXVII. 407.—Annales agronomiques. 1880.
V. Sur l'inversion et sur la fermentation alcoolique du sucre de canne par les moisissures.—C. R. 1878. LXXXVI. 52.—Z. g. Br. 1878. I. 113.
VI. De la fermentation alcoolique avec le Mucor circinelloides.—Annales de chimie et de physique. 5e série. 1878. XIV. 258.

GAYON, U., et DUBOURG, E.,
I. Sur les vins mannités.—Ann. Inst. Past. 1894. VIII. 108.—Ch. C. 1894. I. 787.—K. J. V. 192.—I. (Chap. 64) Ann. Pasteur. 1901. XV. 527.
II. Sur la fermentation alcoolique de la dextrine et de l'amidon.—C. R. 1886. CIII. 885.—C. f. B. I. Abt. 1887. I. 168.
III. De la fermentation de la dextrine et de l'amidon par les Mucors.—Ann. Inst. Past. 1887. I. 534.—C. f. B. I. Abt. 1888. III. 620.
IV. Sur la sécrétion anormale des matières azotées des levures et des moisissures. —C. R. 1886. CII. 978.—Z. g. Br. 1886. IX. 244.

GAYON, U., et DUPETIT, G.
I. Sur un moyen nouveau d'empêcher les fermentations secondaires dans les fermentations alcooliques de l'industrie. —C. R. 1886. CIII. 883.—C. f. B. 1887. I. 232.—Z. g. Br. 1886. IX. 502.
II. Sur la fermentation des nitrates.—C. R. 1882. LXXXXV. 644, 1365.—Ch. C. 1882. 804. 1883. 73.
III. Recherches sur la réduction des nitrates par les infiniment petits.—Nancy. 1886.
IV. Ann. Pasteur. 1901. XV. 527.

GÉDULD, Robert,
I. Über ein neues Enzym: die Glucase. —Journal de la Distillerie française. 1891.—W. f. Br. 1891. VIII. 548.—Ch. C. 1891. II. 323.—K. J. II. 250.
II. W. f. Br. 1891. VIII. 545.

GEISLER, Theodor,
I. Zur Frage über die Wirkung des Lichtes auf Bakterien. — C. f. B. 1892. [XI. 161.—K. J. II. 94.

GELIS, Amédée,
See PELOUZE et GÉLIS.

GENTIL,
I. Z. f. Spiritusindustrie. 1898. XXI. 16.

GEORGIEWICZ, Georg von,
I. Der Indigo vom praktischen und theoretischen Standpunkt dargestellt. Wien. 1892. 6*s.*, bound.

GEORGIEWSKI, Nikolai,
I. Über die Beziehungen des Kwass zum Biere und die diätetische Bedeutung der freien Säuren in diesen Getränken. Dissert. St. Petersburg. 1875.

GÉRARD, E.,
I. Fermentation de l'acide urique par les microorganismes.—C. R. 1896. CXXII. 1019; CXXIII. 185.—Ch. C. 1896. I. 1272.—K. J. VII. 218.
II. Sur une lipase végétale extraite du Penicillium glaucum.—C. R. 1897. CXXIV. 370.—Ch. C. 1897. I. 768.—K. J. VIII. 269.
III. Sur les cholésterines végétales.—C. R. 1892. CXIV. 1544.—Ch. C. 1892. II. 287.
IV. Sur les cholésterines des Cryptogames. —C. R. 1895. CXXI. 723.—Ch. C. 1896. I. 45.—K. J. VI. 62.
V. Sur les cholésterines des végétaux inférieurs. — C. R. 1898. CXXVI. 909.—Ch. C. 1898. I. 949.
VI. Comptes rendus Soc. de Biologie. 1893. 651.
VII. Journ. de pharm. et de chim. 1893. 5th Series. XXVIII. 11.

GÉRARD, E., et DAREXY, P.,
I. Recherches sur la matière grasse de la levure de bière.—Bulletin de la Soc. mycol. de France. 1897. XIII. 183. —Journal de pharm. et de chimie. 1897. [6.] V. 275.—Ch. C. 1897. I. 780.—K. J. VIII. 89.

GERBER, C.,
I. Recherches sur la maturation des fruits charnus.—Annales des sciences naturelles. Botanique. 8e série. 1897. IV. 1.—Has a full antecedent bibliography. —C. R. 1897. CXXIV. 1106, 1160. —Bot. Ztg. 1897. LV. 154, 243, 245.—Ch. C. 1897. II. 43.

GERET, L.,
I. Münch. med. Wochenschrift. 1901. XLVIII. 1836.

GERET, Ludwig, und HAHN, M.,
I. Zum Nachweis des im Hefepresssaft enthaltenen proteolytischen Enzyms.—Ber. d. D. Chem. Ges. 1898. XXXI. 202.—C. f. B. 2. Abt. 1898. IV. 491.—Ch. C. 1898. I. 680.—K. J. IX. 291.
II. Weitere Mitteilungen über das im Hefepresssaft enthaltene proteolytische Enzym.—Ber. d. D. Chem. Ges. 1898. XXXI. 2335.—C. f. B. 2. Abt. 1899. V. 41.—Ch. C. 1898. II. 1272.—K. J. IX. 292.
See also HAHN und GERET.

GERET, L., u. MARTIN, M.,
I. Ber. d. D. Chem. Ges. 1898. XXXI. 202, 2335.

GERSTMANN, H.,
I. Über die Ursache der Gerinnung der Milch bei Gewittern.—Elektrotechnische Zeitschrift. 1896. III. 74.—Ch . C. 1896. II. 388.—K. J. VII. 165.

GESSARD, C.,
I. Fonctions et races du bacille cyanogène (microbe du lait bleu).—Ann. Inst. Past. 1891. V. 737.—C. f. B. 1892. XI. 375.—K. J. II. 108.
II. De la pyocyanie et de son microbe. Thèse de Paris. 1882.
III. Nouvelles recherches sur le microbe pyocyanique.—Ann. Inst. Past. 1890. IV. 88.—C. f. B. 1890. VII. 740.—K. J. I. 33.
IV. Des races du bacille pyocyanique.—Ann. Inst. Past. 1891. V. 65.—C. f. B. 1891. IX. 541.—K. J. II. 107.
V. Sur la fonction fluorescigène des microbes.—Ann. Inst. Past. 1892. VI. 801.—C. f. B. 1893. XIV. 695.—K. J. III. 86.

GEUNS, Ib. van,
I. Über die Einwirkung des sog. Pasteurisierens auf die Milch.—A. f. Hygiene. 1886. III. 464.—Ch. C. 1886. 30.
II. Über das Pasteurisieren von Bakterien.—A. f. Hygiene. 1889. IX. 369.—C. f. B. 1889. VI. 684.—Z. g. Br. 1889. XII. 353.

GEUTHER,
I. Liebig's Ann. 1863. CXXVI. 63.

GIARD, A.,
I. Sur l'infection phosphorescente des Talitres et autres Crustacés.—C. f. B. 1889. VI. 645.
II. Nouvelles recherches sur les bactéries lumineuses pathogènes.—Comptes rendus de la Société de Biologie. Paris. 1890. No. 14.—C. f. B. 1890. VIII. 177.
III. Sur le Crenothrix Kühniana, cause de l'infection des eaux de Lille.—C. R. 1882. LXXXV. 247.

GIBELLI e GRIFFINI,
I. Sul polimorfismo della Pleospora herbarum.—Archivio del Laborat. di Botanica crittogamica. Pavia. 1874. I. 53.

GIBSON, Howard B.,
I. On the Liberation of Nitrogen during the Process of Putrefaction.—Dissert. Leipzig. 1893.—Wollny's Forschungen. 1895. VIII. 106.—Biedermann's Centralblatt. 1895. XXIV. 701.—Ch. C. 1896. I. 125.—K. J. IV. 237.

GILBERT, J. H.,
See LAWES and GILBERT.

GILKINET, A.,
I. Mémoire sur le polymorphisme des Champignons.—Mémoires couronnés et autres mémoires publiés par l'Académie Royale des sciences, etc., de Belgique. 1878. XXVI. 1.

GILLOT, Henri,
I. Sur la fermentation du raffinose par le Schizosaccharomyces Pombe.—Bulletin de la Société belge de Microscopie. 1899. XXV. 29.
II. Die Raffinose als Kohlenhydrat-Nahrungsmittel des Aspergillus niger.—Bulletin de l'Académie Roy. de Belgique. 1889, p. 211.—Ch. C. 1899. II. 129.
III. Bull. de l'Assoc. belge des Chimistes. 1900. 202.
IV. *Ibid.* 1899. 496.
V. *Ibid.* 1902. XVI. 240.
VI. Bull. de l'Acad. Royale de Belgique. 1899. 221.

GILSON, Eugène,
I. La cristallisation de la cellulose et la composition chimique de la membrane cellulaire végétale.—La Cellule. 1893. IX. 397.—Ch. C. 1893. II. 530.—Bot. Ztg. 1893. LI. 309.
II. Recherches chimiques sur la membrane cellulaire des Champignons.—La Cellule. 1894. XI. 7.—Ch. C. 1894. II. 874.
III. De la présence de la chitine dans la membrane cellulaire des Champignons.—C. R. 1895. CXX. 1000.—Ch. C. 1895. I. 1180.
IV. Das Chitin und die Membranen der Pilzzellen.—Ber. d. D. Chem. Ges. 1895. XXVIII. 821.—Ch. C. 1895. I. 1113.

GILSON, G.,
I. On the Affinity of Nuclein for Iron and other Substances.—Report of the British Association for the Advancement of Science. Edinburgh. 1892, p. 778.

GILTAY, E.,
I. Pasteur und die alkoholische Gärung.—Jahrbücher f. wissenschaftl. Botanik. 1896. XXX. 71.

GILTAY, E., und ABERSON, J. H.,
I. Recherches sur un mode de dénitrification et sur le schizomycète qui la produit.—Archives néerlandaises. 1892. XXV. 341.—C. f. B. 1892. XII. 864.—K. J. III. 226.
II. Über den Einfluss des Sauerstoffzutritts auf Alkohol- und Kohlensäurebildung bei der alkoholischen Gärung.—Jahrbücher f. wissenschaftl. Botanik. 1894. XXVI. 543.—K. J. V. 119

GIMEL, G.,
I. Bull. de l'Assoc. de sucr. et de distill. 1905. XXIII. 669.

GIRARD, A. Ch.,
See MÜNTZ et GIRARD.

GIRARD, Aimé,
I. Sur la fermentation panaire.—C. R. 1885. CI. 601.—Ch. C. 1885. 796.
II. Sur la température de cuisson du pain. —C. R. 1893. CXVII. 584.—Ch. C. 1894. I. 89.

GIRARDIN,
I. Note pour servir à l'étude du lait.—C. R. 1853. XXXVI. 753.

GIUNTI, Michele,
I. Sull' azione della luce sulla fermentazione acetica.—Staz. sperim. agrar. ital. 1890. XVIII. 172.—Ch. C. 1890. II. 65.—K. J. I. 139.

GLASER, Fritz,
I. Zur Gallertausscheidung in Rübensäften.—C. f. B. 2. Abt. 1895. I. 879. —Ch. C. 1896. I. 452.

GLASMACHER,
I. Vergiftung durch Hühnereiweiss.—Berl. klin. Wochenschrift. 1886. 666. —C. f. B. 1887. I. 233.

GLAUBITZ, H.,
See TOLLENS und GLAUBITZ.

GLOBIG,
I. Über Bakterienwachstum bei 50 bis 70°. —Z. f. Hygiene. 1888. III. 294.—C. f. B. III. 366.—Ch. C. 1888. 224.
II. Über einen Kartoffelbacillus mit ungewöhnlich widerstandsfähigen Sporen. —Z. f. Hygiene. 1887. III. 322.—Ch. C. 1888. 223.

GODLEWSKI, Emil,
I. Zur Kenntnis der Nitrifikation.—Anzeiger d. Akad. d. Wiss. in Krakau. 1892. 408.—C. f. B. 1893. XIII. 559. —Ch. C. 1895. II. 87. 1896. I. 49; II. 637.—K. J. III. 219.

GODLEWSKI, E., und POLZENIUSZ, F.,
I. Über Alkoholgärung bei der intramolekularen Atmung höherer Pflanzen. —Anzeiger der Krakauer Akademie. 1897. 267.—Bot. Ztg. 2. Abt. 1897. LV. 273.—Ch. C. 1898. I. 65.—K. J. VIII. 47.
II. Intramolekulare Atmung von Samen. —*Ibid.* 1901. 227.—Ch. C. 1901. II. 595.

GOETHE, Rudolf,
I. Fäulniserscheinungen, welche durch Botrytis cinerea hervorgerufen werden. — Mitteilungen über Weinbau und Kellerwirtschaft. 1894. VI. 101.

GOLDBERG, A.,
I. Chemische Untersuchung von schleimigen Wässern, welche aus destilliertem Wasser entstanden sind. — Ber. d. naturw. Ges. Chemnitz. 1893. 56. — Chem. Ztg. 1894. Repert. XVIII. 3. —Ch. C. 1894. I. 390.

GOLDEN, Kathr., and FERRIS, G. C.,
I. The Botanic. Gazette. 1898. XXV. 39.—Abstr. in C. f. B. 2. Abt. 1898. IV. 647.

GOODFELLOW, John,
I. Engl. Patent No. 13722 of 1897.—French Patent No. 269939 of 1897.—Ch. C. 1898. I. 965.—K. J. IX. 103.

GOODSIR, John,
I. History of a Case in which a Fluid periodically ejected from the Stomach contained Vegetable Organisms of an Undescribed Form.—Edinburgh Medical and Surg. Journal. 1842. LVII. 430.

GOPPELSRÖDER,
I. Beiträge zum Studium der Salpeterbildungen.—Poggendorff's Annalen d. Physik u. Chemie. 1862. CXV. 125.

GORINI, Constantin,
I. Studi sperimentali sul latte.—Rivista d'igiene e san. publ. 1892. No. 18.—C. f. B. 1892. XII. 666.—K. J. III. 182.
II. Il fermento coagulante del bacillo prodigioso.—*Ibid.* 1893, p. 549.—K. J. IV. 290.

GORUP-BESANEZ,
I. Weitere Beobachtungen über diastatische und peptonbildende Fermente im Pflanzenreiche.—Ber. d. D. Chem. Ges. 1875. VIII. 1510.

GOSIO, B.,
I. Über Links Milchsäure bildende Vibrionen.—A. f. Hygiene. 1894. XXI. 114. —C. f. B. 2. Abt. 1895. I. 89.—Ch. C. 1894. II. 701.—K. J. V. 239.
II. Azione di alcune muffe sui composti fissi d'arsenico.—Rivista d'Igiene e Sanità pubblica. 1892, p. 201.—K. J. IV. 86.
III. Sul riconoscimento dell' arsenico per mezzo di alcune muffe.—Rivista d'Igiene e Sanità pubblica. 1892, p. 261.—Baumgarten's Jahresbericht. IX. 444.
IV. Zur Frage, wodurch die Giftigkeit arsenhaltiger Tapeten bedingt wird.—Ber. d. D. Chem. Ges. 1897. XXX. 1024.—Ch. C. 1897. I. 1218.
V. Die Arsenikatur der Felle in Hinsicht auf die Prophylaxis gegen Bubonenpest. —Hyg. Rundschau. 1897. VII. 1217.
VI. Archives ital. de Biologie. 1892. XVIII. 253.
VII. Abst. in Bot. Centralbl. 1901. LXXXVII. 131.
See also SCLAVO und GOSIO.

GOTTSTEIN, A.,
I. Über den Einfluss des elektrischen Stromes auf Bakterien.—C. f. B. 1. Abt. 1896. XIX. 602.—K. J. VII. 54.
II. Über die Zerlegung des Wasserstoffsuperoxyds durch die Zellen mit Bemerkungen über eine makroskopische Reaktion für Bakterien.—Virchow's Archiv für patholog. Anatomie. 1893. CXXXIII. 295.—Ch. C. 1894. I. 168. —K. J. IV. 118.

GOUIRAND, G.,
I. Sur la présence d'une diastase dans les vins cassés.—C. R. 1895. CXX. 887.
See also RAVAZ et GOUIRAND.

GRAF, F.,
I. Jahresb. d. Brauer-Akademie München pro 1899–1900, p. 28.
II. 6th Jahresber. d. Lehranstalt u. Versuchsstation München. Brauer-Akademie pro 1899–1900.

GRAUAUG, A., u. KRANZ, J.,
I. Engl. Patent No. 14150 of 1899.—K. J. 1899. X. 138.

GREEN, J. Reynolds,
I. The Soluble Ferments and Fermentation. Cambridge. 1899. 12*s.* 4*d.* German translation by W. Windisch. Berlin. 1901. 16*s.*
II. The Supposed Alcoholic Enzyme in Yeast.—Annals of Botany. 1897. XI. 535.—K. J. VIII. 279.
III. The Alcohol-producing Enzyme of Yeast.—Annals of Botany. 1899. XII. 491.—K. J. X. 319.
IV. Die Enzyme. German translation by Windisch. Berlin. 1901.

GRÉHANT et QUINQUAND,
I. Dosage de solutions étendues de glucose par la fermentation. — C. R. 1888. CVI. 1249.—Z. g. Br. 1888. XI. 262. —C. f. B. 1. Abt. 1888. IV. 264.
II. Sur la respiration de la levure de grains à diverses températures.—C. R. 1888. CVI. 609.—Z. g. Br. 1888. XI. 182. —C. f. B. 1. Abt. 1888. IV. 264.

GRIESSMAYER, Victor,
I. Über das Lupulin.—Dingler's polytechnisches Journal. 1874. CCXII. 67.
II. Die Peptone und das Dickmaischverfahren.—Z. g. Br. 1879. II. 137.
III. Die Proteide der Getreidearten, Hülsenfrüchte und Ölsamen, sowie einiger Steinfrüchte. Heidelberg. 1897.

GRIFFINI,
See GIBELLI e GRIFFINI.

GRIFFITH,
I. Chem. News. 1886. LIII. 28.

GRIFFITHS, A. B.,
I. Sur la matière colorante du Micrococcus prodigiosus.—C. R. 1892. CXV. 321. —K. J. III. 86.

GRIGORIEW,
I. Vergleichende Studien über die Zersetzung des Hühnereiweisses durch Vibrionen.—C. f. B. 1. Abt. 1895. XVII. 885.

GRIJNS,
I. C. f. B. 2. Abt. 1903. XI. 330.

GRIMBERT, L.,
I. Fermentation anaérobie produite par le Bacillus orthobutylicus, ses variations sous certaines influences biologiques.—Ann. Inst. Past. 1893. VII. 353.—Ch. C. 1894. I. 871.—K. J. IV. 241.
II. Action du pneumobacille de Friedländer sur la xylose et l'arabinose.—Comptes rend. de la Société de Biologie de Paris. 1896, p. 191.—K. J. VII. 223.
III. Comptes rendus Soc. de Biologie. 1896. 191, 260.—Ann. Inst. Pasteur. 1896. X. 708.
IV. Ann. Inst. Pasteur. 1896. 192, 684.

GRIMM,
I. Liebig's Ann. 1871. CLVII. 264.

GRIMM, M.,
I. Landw. bakter. Laborat. an Ministerium d. Agricultur St. Petersburg. 1900, p. 18.

GRIMMER, H.,
I. Betrachtungen und Analysen aus dem Grossbetriebe.—Z. g. Br. 1881. IV. 181. —Ber. d. D. Chem. Ges. 1881. XIV. 140.

GROMOW, T., u. GRIGORIEW, O.,
I. Zeits. f. physiol. Chemie. 1904. XLII. 299.

GRÖNLUND, Chr.,
I. Eine neue Torula-Art und zwei neue Saccharomyces-Arten.—Z. g. Br. 1892. XVII. 289.—C. f. B. 1. Abt. 1892. XII. 753.—K. J. III. 141.
II. Z. g. Br. 1892. XV. 281.

GROTENFELT, Gösta,
I. Über rote Milch.—Fortschritte d. Medicin. 1889. VII. 42.—C. f. B. 1889. V. 383.
II. Über die Virulenz einiger Milchsäure-Bakterien. And
III. Über die Spaltung von Milchzucker durch Sprosspilze und über schwarzen Käse. *Ibid.* 121.—C. f. B. 1889. V. 607.
IV. *Ibid.* VII. 131.
V. Milchztg. 1891. 759.

GRUBER, Max,
I. Notiz über die Widerstandsfähigkeit der Sporen von Bacillus subtilis gegen Wasserdämpfe von 100° C.—C. f. B. 1888. III. 576.
II. Eine Methode der Kultur anaërobischer Bakterien.—C. f. B. 1887. I. 367.
III. Die Methoden des Nachweises von Mutterkorn in Mehl und Brot.—A. f. Hygiene. 1895. XXIV. 228.—C. f. B. 2. Abt. 1896. II. 132.—Ch. C. 1895. II. 908.
IV. Arch. f. Hygiene. 1893. XVI. 35.
See also BUCHNER und GRUBER.

GRUBER, Max, und WIENER, Emil,
I. Cholerastudien.—A. f. Hygiene. 1892. XV. 241.—C. f. B. 1893. XIV. 76.—Ch. C. 1893. I. 48.

GRUBER, Th.,
I. Die Arten der Gattung Sarcina.—Arbeiten a. d. Bakteriolog. Institut der Techn. Hochschule zu Karlsruhe. 1895. I., Heft 2. 239.—C. f. B. 2. Abt. 1895. I. 588.

GRÜNFELD, Abraham,
I. Beiträge zur Kenntnis der Mutterkornwirkung.—Arbeiten des Pharmakologischen Institutes Dorpat. 1892. VIII. 108. 1895. XI. u. XII. 295.—Ch. C. 1892. II. 372. 1895. II. 175.

GRÜSS,
I. In Festschrift f. Schwendenen. 1899.
GRÜSS, J.,
I. Z. g. Br. 1904. XXVII. 686, 771.
II. W. f. Br. 1901. XVIII. 336.
III. Ber. d. D. Bot. Ges. 1902. XX. 36.
GUÉGUEN,
I. Bull Soc. Mycol. de France. 1899. XV. 171.
II. *Ibid.* 1898. XIV. 201. 1899. XV. 15.
III. Les Champignons parasites de l'homme et des Animaux. Paris. 1904.
IV. Bull. Soc. Mycol. de France. 1898. XIV. 88.
GUÉRARD,
I. Note sur une altération singulière du pain.—Annales d'Hygiène publique et de Médecine légale. 1843. XXIX. 35.
GUÉRIN, G.,
I. Sur la présence d'un alcaloide dans les vins naturels.—Journal de Pharmacie et de Chimie. 1898. [6.] VII. 323.—Ch. C. 1898. I. 1039.
GUÉRIN, P.,
I. Sur la présence d'un champignon dans l'ivraie (Lolium temulentum L.). — Journal de Botanique. 1898, 230, 384.
GUICHARD, P.,
I. Composition et analyse de la levure.—Bulletin de la Société chimique de Paris. Série III. 1894. XI. 230.—Ch. C. 1894. I. 687.—K. J. V. 115.
GUILLEBEAU, A.,
I. Beiträge zur Lehre von den Ursachen der fadenziehenden Milch. — Landw. Jahrbuch der Schweiz. 1891. V.—C. f. B. 1892. XI. 438.—K. J. II. 185.
GUILLIERMOND, A.,
I. Recherches sur la germination des spores dans le Saccharomyces Ludwigii (Hansen).—Bulletin de la Société mycologique de France. 1903. XIX. 18.
II. C. R. 1901. CXXXII. 175, 1194; CXXXIII. 242.—Ann. de la Soc. Bot. de Lyon. 1903.
IV. Recherches cytologiques s. les levures et quelques moisissures à formes levures. Lyon. 1902. 203.
V. *Ibid.*, p. 211.
VI. Revue génér. botan. 1903. XV. 49.
GUNNING,
I. Ber. d. D. Chem. Ges. 1875. V. 821.
GÜNTHER, Ernst,
I. Beitrag zur mineralischen Nahrung der Pilze.—Erlangener Dissert. 1897.—Bot. Ztg. 2. Abt. 1897. LV. 379.
GÜNTHER, Karl, und THIERFELDER, H.,
I. Bakteriologische und chemische Untersuchungen über die spontane Milchgerinnung. — A. f. Hygiene. 1895. XXV. 164.—C. f. B. 2. Abt. 1896. II. 118.—Ch. C. 1895. I. 295. 1896. I. 268.
HAAS, B.,
I. Über die Bildung von schwefliger Säure bei der Gärung.—Zeitschr. f. Nahrungsmittel-Untersuchung, u.s.w. 1890. III. 241.—Ch. C. 1890. I. 499.—K. J. I. 65.
II. Die Untersuchung des Weines.—Zeitschrift f. Nahrungs Mittel-Untersuchung, u.s.w. 1897. XI. 122, 124.—Ch. C. 1897. I. 1260.
HABERLANDT, Fr.,
I. Die Sojabohne. 1878.
HADDON,
See CAZENEUVE et HADDON.
HAENLEIN, F. H.,
I. Beitrag zur Kenntnis der Wirkung des Kochsalzes auf die Fäulnisbakterien der Haut.—Dingler's polyt. Journal. 1893. CCLXXXVIII. 214.
II. Bakterien auf unseren Gerberrinden und ihre Bedeutung.—Tharander forstliches Jahrbuch. 1893. XLIII. 56.—Deutsche Gerberzeitung. 1892. No. 48. —Ch. C. 1893. II. 700.—K. J. IV. 255.
III. Über die Ursache der sauren Gärung in Gerbebrühen.—Dingler's polytechn. Journal. 1894. CCLXXXXI. 186.—Ch. C. 1894. I. 801.—C. f. B. 2. Abt. 1895. I. 26.—K. J. V. 270.
HAGENMÜLLER,
I. Chem. Ztg. 1902. XXVI. 209.
HAHN, Martin,
I. Das proteolytische Enzym des Hefepresssaftes.—Ber. d. D. Chem. Ges. 1898. XXXI. 200.—Ch. C. 1898. I. 680.—K. J. IX. 290.
II. Ber. d. D. Chem. Ges. 1900. XXXIII. 3555.
III. Z. f. Biologie. 1900. XC. 172.
IV. Münch. med. Wochenschrift. 1902. 595.
See also GERET und HAHN.
HAHN, M., und GERET, L.,
I. Über das Hefe-Endotrypsin.—Zeitschr. f. Biologie. 1900. XL. 117.—C. f. B. 2. Abt. 1901. VII. 394.—Ch. C. 1900. II. 641.
HAIENKE u. KURTZ,
I. Liebig's Ann. 1871. CLVII. 270.
HAITINGER, Ludwig,
I. Über das Vorkommen organischer Basen im käuflichen Amylalkohol.—Sitzgsber. d. Wiener Akademie. Mathem.-naturh. Klasse. 1882. LXXXVI. 2. Abt. 608.
HALLIBURTON, W. D.,
I. A Text-book of Chemical Physiology and Pathology. 1891. London.
HALLIER, Ernst,
I. Gärungserscheinungen. Leipzig. 1867.
II. Die Hefe der Alkoholgärung insbesondere der Biergärung. Weimar. 1896. —Z. g. Br. 1896. XIX. 609.—K. J. VII. 109.

HAMMARSTEN, Olaf,
I. Über die Milchgerinnung und die dabei wirkenden Fermente der Magenschleimhaut.—Upsalas läkareförenings förhandlingar. 1872. VIII. 63.—Maly's Jahresber. f. Tierchemie. 1872. II. 118.
II. Untersuchungen von Kéfir.—Upsalas läkareförenings förhandlingar. 1886. XXI. — Biedermann's Centralblatt f. Agriculturchemie. 1888. XVII. 413.—Jahresber. f. Tierchemie. 1886. XVI. 163.
III. Zur Kenntnis der Nucleoproteide.—Z. f. physiolog. Chemie. 1894. XIX. 19.—Ch. C. 1894. I. 638.

HAMMERL, Hans,
I. Über die in rohen Eiern durch das Wachstum von Cholera vibrionen hervorgerufenen Veränderungen. — Z. f. Hygiene. 1894. XVIII. 153.—C. f. B. 1894. XVI. 787.—Ch. C. 1894. II. 701.

HANAUSEK, Thomas Franz,
I. Vorläufige Mitteilung über den von A. Vogl in der Frucht von Lolium temulentum entdeckten Pilz.—Ber. d. D. Bot. Ges. 1898. XVI. 203.—C. f. B. 2. Abt. 1899. V. 365.

HANKIN, E. H.,
I. Immunity produced by an Albumose isolated from Anthrax Cultures.—British Med. Journal. 1889, p. 810.—C. f. B. 1889. VI. 617.

HANRIOT,
I. Sur un nouveau ferment du sang.—C. R. 1896. CXXIII. 753.—Ch. C. 1897. I. 64.

HANSEN, Adolf,
I. Die Verflüssigung der Gelatine durch Schimmelpilze.—Flora. 1889. LXXII. 88.—Bot. Centralblatt. 1889. XL. 74.—Z. g. Br. 1889. XII. 488.
II. Über Fermente und Enzyme.—Arbeiten d. botan. Instituts zu Würzburg. 1885. III. 253.—Z. g. Br. 1885. VIII. 317.

HANSEN, Emil Christian,
I. Hypothèse de Horváth.—Compte rendu des travaux du laboratoire de Carlsberg. 1879. I. 94.
II. Contributions à la connaissance des organismes qui peuvent se trouver dans la bière et le moût de bière et y vivre.—*Ibid.* 1879 and 1882. I. 49, 197.—Z. g. Br. 1880. III. 277. 1882. V. 208.
III. Untersuchungen aus der Praxis der Gärungsindustrie. Erstes Heft. 3. Ed. 1895; Zweites Heft. 1892. München. Oldenbourg.—Z. g. Br. 1884. VII. 272.
IV. Methode zur Analyse des Brauwassers in Rücksicht auf Mikroorganismen.—Z. g. Br. 1888. XI. 1.—C. f. B. 1888. III. 377.—Compare Untersuchungen a. d. Praxis der Gärungsindustrie. 2. Heft.
V. Ein paar für die Brauerei wichtige Punkte.—Z. g. Br. 1890. XIII. 4.
VI. Mycoderma aceti et Myc. Pasteurianum. Compte rendu, etc., de Carlsberg. 1879. I. 96.
VII. Recherches sur les bactéries acétifiantes.—*Ibid.* 1894. III. 182. 1900. V. 39.—C. f. B. 2. Abt. 1896. I. 31. 1901. VII. 439.—K. J. V. 272; XI. 299.
VIII. Action des ferments alcooliques sur les diverses espèces de sucre.—Compte rendu, etc., de Carlsberg. 1887. II. 143.—Transl. in Z. g. Br. 1888. XI. 401.
IX. Sur le Saccharomyces apiculatus et sa circulation dans la nature.—Compte rendu, etc., de Carlsberg. 1881. I. 159; transl. in Z. g. Br. 1881. IV. 449.
X. Vorläufige Mitteilungen über Gärungspilze.—Botan. Centralblatt. 1885. XXI. 181.—Reproduced in Z. g. Br. 1885. VIII. 147.
XI. Nouvelles recherches sur la circulation du Saccharomyces apiculatus dans la nature.—Annales des sciences naturelles. Botanique. 7e sér. 1890. XI. 185.—Transl. in Z. g. Br. 1890. XIII. 413.—Ch. C. 1890. II. 757.—K. J. I. 41.
XII. Les ascospores chez le genre Saccharomyces.—Compte rendu, etc., de Carlsberg. 1883. II. 13.—Transl. in Z. g. Br. 1883. VI. 310.
XIII. Sur les Torulas de M. Pasteur.—Compte rendu, etc., de Carlsberg. 1883. II. 47.—Transl. in Z. g. Br. 1883. VI. 460.
XIV. Maladies provoquées dans la bière par des ferments alcooliques.—Compte rendu, etc., de Carlsberg. 1883. II. 52.—Transl. in Z. g. Br. 1883. VI. 477.
XV. Méthodes pour obtenir des cultures pures de Saccharomyces et de microorganismes analogues.—Compte rendu, etc., de Carlsberg. 1886. II. 92.—Transl. in Z. g. Br. 1886. IX. 270.
XVI. Les voiles chez le genre Saccharomyces.—Compte rendu, etc., de Carlsberg. 1886. II. 106.—Transl. in Z. g. Br. 1886. IX. 374.
XVII. Sur la germination des spores chez les Saccharomyces.—Compte rendu, etc., de Carlsberg. 1891. III. 44.—Transl. in Z. g. Br. 1891. XIV. 405.—C. f. B. 1891. IX. 663. 1893. XIII. 101.—K. J. II. 35.
XVIII. Kritische Untersuchungen über einige von Ludwig und Brefeld beschriebene Oïdium und Hefenformen.—Bot. Ztg. 1892. L. 312.—C. f. B. 1. Abt. 1892. XII. 145.—K. J. III. 42.
XIX. Über die neuen Versuche, das Genus Saccharomyces zu streichen.—C. f. B. 1. Abt. 1893. XIII. 16.—K. J. IV. 44.
XX. Anlässlich Juhler's Mitteilung über einen saccharomyces bildenden Aspergillus.—C. f. B. 2. Abt. 1895. I. 65.—K. J. VI. 40.

XXI. Neue Untersuchungen über Alkoholgärungspilze.—Ber. d. D. Bot. Ges. 1884. II. S. XXXII.—Z. g. Br. 1884. VII. 471.

XXII. Nogle Undersögelser over Agaricineernes Biologi.—Hospitalstidende. 1897. 1109.—Botan. Centralblatt. 1898. LXXIV. 114.

XXIII. Über Saccharomyces apiculatus.—Hedwigia. 1880. 75.

XXIV. Sur la vitalité des ferments alcooliques et leur variation dans les milieux nutritifs et à l'état sec.—Compte rendu, etc., de Carlsberg. 1898. IV. 93.—Z. g. Br. 1898. XXI. 624.—C. f. B. 2. Abt. 1898. IV. 862.—K. J. IX. 89.

XXV. Observations sur les levures de bières.—Annales de micrographie. 1888. I. 11.

XXVI. Sur la production de variétés chez les Saccharomyces. — Ann. de micrographie. 1889. II. 214.—C. f. B. 1. Abt. 1890. VII. 795.

XXVII. Biologische Untersuchungen über Mist bewohnende Pilze.—Bot. Ztg. 1. Abt. 1897. LV. 111.

XXVIII. Neue Untersuchungen über die Sporenbildung bei den Saccharomyceten. —C. f. B. 2. Abt. 1899. V. 1.—K. J. X. 21.

XXIX. Über die in dem Schleimflusse lebender Bäume beobachteten Mikroorganismen.—C. f. B. 1. Abt. 1889 V. 632.—Z. g. Br. 1889. XII. 253.

XXX. Sur l'influence que l'introduction de l'air atmosphérique dans le moût qui fermente exerce sur la fermentation.—Compte rendu, etc., de Carlsberg. 1879. I. 88.

XXXI. Über die zymotechnische Analyse der Mikroorganismen der Luft.—Prager Brauer- u. Hopfenzeitung. 1888. 223. —Z. g. Br. 1888. XI. 471.

XXXII. Recherches comparatives sur les conditions de la croissance végétative et le développement des organes de reproduction des levures et des moisissures de la fermentation alcoolique.—Compte rendu, etc., de Carlsberg. 1902. V. 68.

XXXIII. Über Hefe und Hefereinzucht. —Allgem. Zeitschrift f. Bierbrauerei und Malzfabrikation. 1887. XVI. 518.—C. f. B. 1887. II. 118.

XXXIV. Qu'est-ce que la levure pure de M. Pasteur ?—Compte rendu, etc., de Carlsberg. 1891. III. 24.—Transl. in Z. g. Br. 1891. XIV. 305.—C. f. B. 1. Abt. 1891. X. 557.—K. J. II. 143.

XXXV. Unters. u. d. Praxis d. Gärungsindustrie. 3rd Ed. 1895.

XXXVI. W. f. Br. 1887. IV. 378.

XXXVII. Compte rendu, etc., de Carlsberg. 1883. II. 41.

XXXVIII. *Ibid.* 1886. II. 119.

XXXIX. *Ibid.* 1886. II. 130.

XL. *Ibid.* 1888. II. 189.

XLI. C. f. B. 1889. V. 664.

XLII. Medd. fra Carlsb. Laborat. 1892. III. 200; and Unters. aus d. Praxis d. Gärungsind. 1895. Heft I. 79.

XLIII. Annals of Botany. 1895. IX. 549.

XLIV. Compte rendu, etc., de Carlsberg. 1900. V. 1.

XLV. C. f. B. 2. Abt. 1905. XV. 353.

XLVI. Ann. de Micrographie. 1888. I. 49. —Compte rendu, etc., de Carlsberg. 1888 [? 1887]. II. 143.

XLVII. C. f. B. 1889. V. 638.

XLVIII. Compte rendu, etc., de Carlsberg. 1902. V. 68.

XLIX. C. f. B. 1904. 2. Abt. XII. 529.

L. Medd. fra Carlsberg Laborat. 1879. I. Pl. II.

LI. Compte rendu, etc., de Carlsberg. 1879. I. 72.

LII. *Ibid.*, p. 81.

LIII. *Ibid.* 1882 [1879]. I. 47.

LIV. Allgem. Brauer. u. Hopfen Ztg. 1887. XVII. 1109.

LV. Compte rendu, etc., de Carslberg. 1902. V. 92.

LVI. C. f. B. 2. Abt. 1905. XIV. 545.

LVII. *Ibid.* 1905. XIV. 547.

LVIII. Compte rendu, etc., de Carlsberg. 902. V. 68.

LIX. Medd. fra Carlsberg Laborat. 1879. I. 185, 226.

LX. Compte rendu, etc., de Carlsberg. 1882. I. 203.

LXI. Medd. fra Carlsberg Laborat. 1881. I. 322.

LXII. A. des Sci. Nat. Bot. 1890. XI. 185.

LXIII. C. f. B. 2. Abt. 1903. X. 1. 1905. XIV. 545.

LXIV. Z. f. d. Ges. Br. 1888. XI. 417.

LXV. Untersuchungen a. d. Praxis d. Gärungsindustrie. München u. Leipzig. 1888. Heft I. 62.

HANSGIRG, A.,

I. Über neue Süsswasser- und Meeres-Algen und Bakterien, etc.—Sitzgsber. d. Kgl. Böhm. Ges. d. Wissenschaften in Prag. 1890.—K. J. I. 19.

HANTKE, E.,

I. Inwieweit stimmen die Gärversuche im kleinen (Laboratoriumsversuche) mit den Gärungen in der Praxis überein ?—Der amerik. Bierbrauer. 1896. XXIX. 213.—Z. g. Br. 1896. XIX. 458.—C. f. B. 2. Abt. 1896. II. 359.—Ch. C. 1896. II. 273.—K. J. VII. 112.

See also WAHL u. HANTKE.

HANUŠ, Jos., u. STOCKÝ, Alb.,

I. Über die chemische Einwirkung der Schimmelpilze auf die Butter.—Zeitschrift f. Untersuchung der Nahrungs- u. Genussmittel. 1900. III. 606.—Ch. C. 1900. II. 922.—C. f. B. 2. Abt. 1901. VII. 29.—K. J. XI. 235.

HAPP, C.,
I. Bakteriologische und chemische Untersuchungen über die chleimige Gärung. Basel. 1893. This also gives the antecedent literature.—Z. f. B. 1893. XIV. 175.—Ch. C. 1894. I. 161.—Z. g. Br. 1893. XVI. 360.—K. J. IV. 247.

HARDEN, A.,
I. Ber. d. D. Chem. Ges. 1903. XXXVI. 715.

HARDEN, Arthur, and ROWLAND, Sydney,
I. Autofermentation and Liquefaction of Pressed Yeast.—Journal of the Chemical Society of London. 1901. LXXIX. 1227.—Ch. C. 1901. II. 1357.

HARDEN, A., u. YOUNG,
I. Ber. d. D. Chem. Ges. 1904. XXXVII. 1052.
II. Journ. of Physiology. 1905. XXXII. No. 1.
III. Proc. Chem. Soc. 1905. XXI. 189.

HARNACK, E.,
See SCHMIEDEBERG u. HARNACK.

HARRIOTT et CAMUS,
I. C. R. 1897. CXXIV. 235.

HARRISON, Francis C.,
I. Foul Brood of Bees.—Ontario Agricultural College. Bulletin 112. Toronto. 1900.—C. f. B. 2. Abt. 1900. VI. 421. —Gives the earlier literature.
II. C. f. B. 2. Abt. 1902. IX. 206.

HARTIG, Robert,
I. Der echte Hausschwamm. Berlin. 1885.—2nd Ed., edited by C. Freiherrn von Tubeuf. Berlin. 1902. Springer.

HARTMANN, M.,
I. W. f. Br. 1903. XX. 113.

HARZ, C. O.,
I. Grundzüge der alkoholischen Gärung. München. 1877.—Z. g. Br. 1878. I. 52.
II. Über das Vorkommen von Lignin in Pilzen.— Botan. Centralblatt. 1885. XXIII. 371. 1886. XXV. 386.
III. Sitzungsb. d. Bot. Vereins in München. 1890.
IV. Bot. Centralbl. 1890. XLI. 378.
V. Sitz. d. Ges. f. Morphologie u. Biologie. München. 1901. XVI. 36.

HATCH and ROW,
I. The Lancet. 1900. Dec. 1.

HATTORI,
I. Journ. Coll. of Sci. Imp. Univ. Tokyo. XV. 371.

HAUBNER,
I. Über die fehlerhafte Beschaffenheit der Kuhmilch im Allgemeinen und die blaue Milch insbesondere.—Magazin f. d. gesamte Tierheilkunde. Berlin. 1852. 1, 129.

HAUSER, Gustav,
I. Über Fäulnisbakterien und deren Beziehungen zur Septikämie. Leipzig. 1885. F. C. Vogel.
II. Über Verwendung des Formalins zum Konservieren von Bakterienkulturen.—Münchner medizin. Wochenschrift. 1893. XL. 567, 655.—C. f. B. 1893. XIV. 290, 468.—Ch. C. 1893. II. 691.—K. J. IV. 113.
III. Über das Vorkommen von Mikroorganismen in den lebenden Geweben gesunder Tiere. — Archiv. f. experim. Pathologie und Pharm. 1885. XX. 162.—C. f. B. 1887. I. 230.

HAUTEFEUILLE, P., et PERREY, A.,
I. Contribution à l'étude des levures.—C. R. 1894. CXVIII. 589.—K. J. V. 184.

HAYDUCK, M.,
I. Über den Einfluss der Hopfenharze auf die Biergärung.—W. f. Br. 1892. IX. 617.—Z. g. Br. 1892. XV. 299.—C. f. B. XII. 663.—K. J. III. 117.
II. Über Milchsäuregärung.—W. f. Br. 1887. IV. 285.—C. f. B. 1887. II. 34.—Ch. C. 1887. 1042, 1165.
III. Über den Einfluss des Alkohols auf die Entwicklung der Hefe.—Z. f. Spiritusind. 1882. V. 183.—Reprinted in Z. g. Br. 1882. V. 287.
IV. Über die Entwicklung der Hefe in Nährlösungen von verschiedenem Stickstoff-Gehalt.—Z. f. Spiritusind. 1881. IV. 173.—Reprinted in Z. g. Br. 1881. IV. 287.
V. Über das Degenerieren der Hefe in der Brauerei. — W. f. Br. 1884. I. 345.
VI. Über die Regenerierung der Bierhefe. —*Ibid.* 1884. I. 697.
VII. Z. f. Spiritusind. 1880. III. 174.
VIII. *Ibid.* 1881. IV. 341.
IX. W. f. Br. 1885. II. 267.
X. *Ibid.* 1887. IV. 397.
XI. *Ibid.* 1888. V. 937.

HAYDUCK, M., und DELBRÜCK, M.,
I. Bemerkung zu obiger Abhandlung (von Ad. Mayer) [VI.].—Z. f. Spiritusind. 1880. III. 214.—Z. g. Br. 1880. III. 514.

HEBEBRAND, A.,
I. Über die Veränderungen des Brotes beim Schimmeln.—D. Landw. Versuchsstationen. 1893. XLII. 421.—Hygien. Rundschau. 1892. II. 1057.—Ch. C. 1893. I. 223. 1896. I. 53.

HEIDEN, E.,
I. Über Stallmistversuche. — Landwirtschaftl. Vereinsschrift des Baltischen Centralvereins. 1887. 216, 235.—Ch. C. 1888. 633, 1122.

HEIDENHAIN, M.,
I. Kern und Protoplasma. — Festschrift für Kölliker. 1892. 118. (Also in pamphlet form. Leipzig. Engelmann.)

HEIM, Carl,
I. Über das Vorkommen von Furfurol im Bier.—Z. g. Br. 1898. XXI. 155, 176. —Ch. C. 1898. I. 1082.—K. J. IX. 163.

II. Über das Vorkommen und den Nachweis des Furfurols im Bier.—Z. g. Br. 1898. XXI. 148.—Ch. C. 1898. II. 146.—K. J. IX. 163.

Heim, L.,
I. Die Neuerungen auf dem Gebiete der bakteriologischen Untersuchungsmethoden seit dem Jahre 1887.—C. f. B. 1891. X. 260.
II. Zählebige Keime in Gelatine.—C. f. B. 1893. XIII. 649.—K. J. IV. 110.
III. Versuche über blaue Milch.—Arbeiten a. d. K. Gesundheitsamte. 1889. V. 518.—C. f. B. 1890. VIII. 46.—Ch. C. 1889. II. 1029.
IV. Über das Verhalten der Krankheitserreger der Cholera, des Unterleibstyphus und der Tuberkulose in Milch, Butter, Molken und Käse.—Arbeiten a. d. K. Gesundheitsamte. 1889. V. 294.—C. f. B. 1890. VII. 152.

Heimann, R.,
See Eckenroth und Heimann.

Heine, L.,
I. Die Mikrochemie der Mitose, zugleich eine Kritik mikrochemischer Methoden. —Z. f. physiolog. Chemie. 1896. XXI. 494.—Ch. C. 1896. I. 1022.
II. Über die Molybdänsäure als mikroskopisches Reagens.—*Ibid.* 1897. XXII. 132.—Ch. C. 1896. II. 404.

Heintz, W.,
I. Über die Ursache der Koagulation des Milchkaseins durch Lab und über die sogen. amphotere Reaktion.—Journal f. prakt. Chemie. 1872. VI. 374.

Heinze, B.,
I. C. f. B. 2. Abt. 1904. XII. 43. 1905. XIV. 9.
II. Ann. Mycologici. 1903. I. 344.—C. f. B. 2. Abt. 1904. XII. 180. 1905. XIV. 9.
III. C. f. B. 2. Abt. 1902. VIII. 553.—Z. f. Hygiene. 1904. XLVI. 296.

Heinze, B., u. Cohn, E.,
I. Z. f. Hygiene. 1904. XLVI. 286.

Heinzelmann, G.,
I. Ein Beitrag zur Schaumgärungs Frage bei Reinhefe Rasse II.—Z. f. Spiritusind. 1895. XVIII. 190, 207.—K. J. VI. 177, 178.
II. Beiträge zur Vergärung von Melassemaischen.—Z. f. Spiritusind. 1889. XII. 246. — Osterr.-ung. Zeitschrift f. Zuckerindustrie u. Landwirtschaft. 1889. XVIII. 509.—K. J. V. 157.
III. Versuche mit einer Weissbierreinzuchthefe.—Z. f. Spiritusind. 1890. XIII. 133.—Ch. C. 1890. I. 1036.
IV. Verhalten verschieden ernährter Hefen in ihrer Gärwirkung. Diastase als Hefenahrungsmittel.—Z. f. Spiritusind. 1897. XX. 296.—Ch. C. 1898. II. 146.—K. J. VIII. 95.
V. Die Zerstörung der Diastase während der Gärung.—Z. f. Spiritusind. 1898. XXI. 357.—K. J. IX. 283.
VI. Z. f. Spiritusind. 1882. V. 458.

Hellriegel, Hermann,
I. Über die Beziehungen der Bakterien zu der Stickstoffernährung der Leguminosen.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. D. Reiches. 1886. 863.—O. f. B. 1887. I. 133.

Helm, Otto,
I. Über Monas prodigiosa und den von ihr erzeugten Farbstoff. — Archiv der Pharmacie. 1875. [3.] VI. 19.—Ch. C. 1875. 118.
II. Über die chemischen Bestandteile der Auswitterungen von Ziegelsteinmauern (Mauerfrass) und die damit verbundene Salpeterbildung.—Schriften der naturforschenden Ges. zu Danzig. 1894. [2.] Bd. viii. Heft 3.—Ch. C. 1894. I. 1084.—K. J. V. 264.

Helmholtz, H.,
I. Müller's Archiv. 1843. X. 453.

Hemmer, M.,
I. Experimentelle Studien über die Wirkung faulender Stoffe auf den tierischen Organismus. — Gekrönte Preisschrift. München. 1866.

Henius, L.,
I. Populäre Vorlesungen über Reinhefe.—American Brewer's Review. 1895, p. 449.—K. J. VI. 172.

Henius, Max,
See Wahl und Henius.

Henneberg, W.,
I. Die Brennereihefen Rasse II. und Rasse XII.—Z. f. Spiritusind. 1903. XXVI. 91.
II. Z. f. Spiritusind. 1902. XXV. 378.
III. W. f. Br. 1902. XIX. 781.
IV. *Ibid.* 1900. XVII. 633.
V. Z. f. Spiritusind. 1904. XXVII. 96.
VI. *Ibid.* 1902. XXV. 553.
VII. W. f. Br. 1903. XX. 178.
VIII. *Ibid.* 1900. XVII. 113.

Henninger,
I. C. R. 1882. XCV. 94.

Hennings, P.,
I. Der Hausschwamm und die durch ihn und andere Pilze verursachte Zerstörung des Holzes. Berlin. 1891. A. Seydel. 8*d*.—Zeitschr. f. Pflanzenkrankheiten. 1891. I. 125.
II. Hedwigia. 1895. XXXIV. 86.
III. Verhandl. d. Botan. Ver. d. Mark Brandenburg. 1898. XL. 173.

Henrici, H.,
I. Beiträge zur Bakteriologie des Käses. —Baseler Dissertat. 1894.—C. f. B. 2. Abt. 1896. II. 40, 245.—Ch. C. 1898. I. 790.—K. J. V. 244.

Henry, J.,
I. Ann. de brasserie et de distillerie. 1902. 129.—Abst. in W. f. Br. 1902. XIX. 325.

HERAEUS, Wilhelm,
I. Über das Verhalten der Bakterien im Brunnenwasser, sowie über reduzierende und oxydierende Eigenschaften der Bakterien. Dissert. Berlin.—Z. f. Hygiene. 1886. I. 193.

HERFELDT, E.,
See BURRI, HERFELDT und STUTZER.

HÉRISSEY, H.,
I. Comptes rendus Soc. de Biologie. 1896. XLVIII. 915.
II. *Ibid.*, p. 640.
III. Bull. Soc. Mycol. de France. 1899. XV.
IV. C. R. 1901. CXXXIII. 49.
See BOURQUELOT et HÉRISSEY.

HERON, J.,
I. Über die verschiedenen Methoden der Konservierung der Hefe.—Diary for the Brewing Room. 1896.—W. f. Br. 1896. XIII. 163.—Ch. C. 1896. I. 759.—K. J. VII. 108.

HERSELIN, M.,
See RIETSCH et HERSELIN.

HÉRY, M.,
I. Sur une fermentation visqueuse de l'encre. — Annales de Micrographie. 1891. IV. 13.—C. f. B. 1892. XI. 690.—K. J. II. 223.

HERZ, Fr. Josef,
I. Die Bedeutung der Bakteriologie für die Käsebereitung.—Mitteilungen des landw. Vereins im Allgäu. 1894. Heft 5.
See also BURSTERT u. HERZ.

HERZ, Josef,
I. Über schweflige Säure im Biere.— Repertorium der analyt. Chemie. 1885. V. 58.

HERZFELD, Alexander,
I. Das Auftreten rotfärbender Pilze im Rohzucker.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1891. 663.—C. f. B. 1892. XII. 661.—K. J. II. 225.
II. Über Hefe-Untersuchungen und Unterscheidung der Bierhefe und Presshefe. —Spirituszeitung. 1895. 239.—K. J. VI. 166.

HERZFELD, A., und PAETOW, U.,
I. Anwendbarkeit von Fluorverbindungen zur Verhinderung der Invertzuckerbildung. — Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1891. 678.—C. f. B. 1893. XIII. 191.—K. J. II. 225.

HERZOG, R. O.,
I. Z. f. physiolog. Chemie. 1903. XXXVII. 149.
II. *Ibid.*, p. 383.
III. *Ibid.*, p. 381.

HESS, Friedrich,
I. Vergärung von Saccharose durch die Hefen Saaz, Frohberg und Logos unter verschiedenen Ernährungsbedingungen. Dissert. Erlangen. 1897.—K. J. VIII. 105.

HESS, H.,
I. Über die Gärungsfähigkeit des Milchzuckers.—Bulletin scient. de l'Acad. de St.-Pétersbourg. 1837. II. 126.—Poggendorff's Annalen. 1837. XXXXI. 194.

HESS und BORGEAUD,
I. Eine kontagiöse Euterentzündung, gelber Galt genannt [Mastitis catarrhalis infectiosa].—Schweizer Archiv f. Tierheilkunde. 1888. XXX. 157.—Baumgarten's Jahresbericht. 1888. IV. 33.

HESS, E., SCHAFFER, F., u. LANG, M.,
I. Über die Wirkungen des Glaubersalzes auf die Beschaffenheit des Euters und der Milch beim Rindvieh. — Landw. Jahrbuch d. Schweiz. 1894. VII. 210. —Ch. C. 1894. II. 486.

HESSE, W.,
I. Über die gasförmigen Stoffwechselprodukte beim Wachstum der Bakterien. —Z. f. Hygiene. 1893. XV. 17.—C. f. B. 1. Abt. 1893. XIV. 730.—K. J. IV. 79.

HESSENLAND, Fritz,
I. Über die Zusammensetzung des Hefegummis. — Zeitschr. d. Vereins f. d. Rübenzucker-Industrie des Deutschen Reichs. 1892. XXXXII. 671.—Ch. C. 1892. II. 572.—K. J. III. 67.
See also TACKE, IMMENDORF, HESSENLAND, SCHÜTTE und MINSSEN.

HEST, VAN, J. J.,
I. W. f. Br. 1904. XXI. 1.
II. Z. g. Br. 1903. XXVI. 806, 808.

HEYROTH, Anton,
I. Über den Reinlichkeitszustand des künstlichen Eises.—Arbeiten. a. d. K. Gesundheitsamte. 1888. IV. 1.—C. f. B. 1888. IV. 673.

HIEPE, W.,
I. Studien über die Isomaltose und die Amyloine. — The Country Brewer's Gazette. 1893.—W. f. Br. 1894. XI. 28 *et seq.*—Ch. C. 1894. I. 417.—K. J. V. 137.
II. J. Fed. Inst. Brewing. 1895. I. 288.

HIERONYMUS, Georg,
I. Über die Organisation der Hefezellen. —Ber. d. D. Bot. Ges. 1893. XI. 176.—Ch. C. 1893. II. 215. This has the illustrations.—K. J. IV. 50.

HILGER, A.,
I. Förschungsberichte u. Lebensmittel. 1894. I. 132.

HILL, C.,
I. Proc. Chem. Soc. 1898. 156.—Trans. Chem. Soc. LXXIII. 634.
II. Ber. d. D. Chem. Ges. 1901. XXXIV. 1380.
III. Chem. Ztg. 1903. XXVII. 391.

HILLER,
I. Proc. Indiana Acad. Sci. 1901. 272. —Abst. in Bot. Centralbl. 1903. XCII. 333.

Hill-Jones, Th., u. Kressel, E.,
I. Engl. Patents Nos. 15145 and 18714 of 1897.

Hiltner, Lorenz,
I. Vermögen auch Nichtleguminosen freien Stickstoff aufzunehmen ?—D. Landw. Versuchsstationen. 1894. XXXXV. 155.—C. f. B. 2. Abt. 1895. I. 198.—Ch. C. 1894. II. 707.—K. J. V. 259.
II. Über die Assimilation des freien atmosphärischen Stickstoffs durch in oberirdischen Pflanzenteilen lebende Mycelien. —C. f. B. 2. Abt. 1899. V. 831.—Ch. C. 1900. I. 212.
See also Nobbe und Hiltner, Nobbe, Hiltner u. Schmid, Nobbe, Schmid, Hiltner u. Hotter.

Hjort, Johann,
I. Neue eiweissverdauende Enzyme.—Centralblatt f. Physiologie. 1896. X. 192.—Ch. C. 1896. II. 390.

Höhnel, F. von,
I. Sitz. Kais. Akad. Wiss. Wien, Math.-naturw. Kl. 1902. CXI. 1036.

van 't Hoff, J. H.,
I. Die Lagerung der Atome im Raum. Braunschweig. Vieweg.

Hoffmann, F.,
I. Wie gross ist die Zahl der Mikroorganismen auf dem Getreide unter verschiedenen Bedingungen.—W. f. Br. 1896. XIII. 1153.—K. J. VII. 67.

Hoffmann, Hermann,
I. Mykologische Studien über die Gärung. —Bot. Ztg. 1860. XVIII. 49.
II. Untersuchungen über die Keimung der Pilzsporen. — Jahrbücher für wissenschaftl. Botanik. 1860. II. 267.
III. Über Bakterien. — Bot. Ztg. 1869. XXVII. 233.—Ann. des sciences naturelles. Botanique. 1869. XI. No. 1.—Compare C. R. 1870. LXXI. 150.
IV. Zur Naturgeschichte der Hefe.—Karsten's Botan. Untersuchungen. 1867. I. 341.
V. Mykologische Berichte. 1890. I. 30.
VI. Mitt. d. D. Ges. f. Natur. u. Völkerkunde Ostasiens. 1874. Heft 6, p. 8.
VII. Bot. Ztg. 1869. 309.

Hoffmann, Max,
I. Ein Beitrag zur Translokation des Kupfers beim Keltern gekupferter Trauben.—C. f. B. 2. Abt. 1898. V. 369.—Ch. C. 1898. II. 148.

Hoffmeister, W.,
I. Die Rohfaser und einige Formen der Cellulose.'— Landwirtsch. Jahrbücher. 1888. XVII. 239.—Ch. C. 1888. 1211.

Hofmeister, Franz,
I. Die wirksamen Bestandteile des Taumellolchs.—Archiv f. experim. Pathologie u. Pharmakologie. 1892. XXX. 202. —Ch. C. 1892. II. 657.

Holm, J. C.,
I. Z. g. Br. 1889. XII. 301.

Holm, Justus Chr.,
I. Analyses biologiques et zymotechniques de l'eau destinée aux brasseries.—Compte rendu des travaux du laboratoire de Carlsberg. 1892. III. 107.—Z. g. Br. 1893. XVI. 78.—C. f. B. 1893. XIII. 193.—K. J. III. 131.
II. Die Vorrichtungen in der Brauerei zur Kühlung und Lüftung der Würze.—Z. g. Br. 1887. X. 449.
III. Über die Aufbewahrung der Hefe in Saccharoselösung. — C. f. B. 2. Abt. 1896. II. 313.—Ch. C. 1896. II. 251. —K. J. VII. 105.
IV. Sur les méthodes de culture pure et spécialement sur la culture sur plaques de M. Koch et la limite des erreurs de cette methode.—Compte rendu, etc., de Carlsberg. 1891. II. 1.—Z. g. Br. 1891. XIV. 458.—C. f. B. 1892. XI. 576.—K. J. II. 14.

Holm, J. Chr., und Poulsen, S. V.,
I. Jusqu'à quelle limite peut-on, par la méthode de M. Hansen, constater une infection de "levure sauvage" dans une masse de levure basse de Saccharomyces cerevisiæ.—Compte rendu des travaux du laboratoire de Carlsberg. 1886. II. 88.—Z. g. Br. 1886. IX. 241.—C. f. B. 1. Abt. 1887. I. 201.
II. *Ibid.* II.—*Ibid.* 1888. II. 137.—Z. g. Br. 1888. XI. 381.—C. f. B. 1. Abt. 1888. IV. 359.

Holschewnikoff,
I. Über die Bildung von Schwefelwasserstoff durch Bakterien.—Fortschritte der Medizin. 1889. VII. 201. — C. f. B. 1889. VI. 14.—Ch. C. 1889. I. 595.

Holtz, Wilhelm,
I. Beitrag zur Kenntnis der Baumflüsse und einiger ihrer Bewohner.—C. f. B. 2. Abt. 1901. VII. 113.

Holzner, Georg,
I. Dr. Elsner's Ansichten über Bier-Analysen.—Z. g. Br. 1878. I. 481.

Hoppe-Seyler, Felix,
I. Über Gärung der Cellulose mit Bildung von Methan und Kohlensäure. — Z. f. physiolog. Chemie. 1886. X. 201, 401. —Ch. C. 1886. 458, 775.
II. Über Chitin und Cellulose.—Ber. d. D. Chem. Ges. 1894. XXVII. 3329. 1895. XXVIII. 82.—Ch. C. 1895. I. 393, 435.
III. Über Huminsubstanzen, ihre Entstehung und ihre Eigenschaften.—Z. f. physiolog. Chemie. 1889. XIII. 66.
IV. Über die chemische Zusammensetzung des Eiters.—Medic.-chem. Untersuchungen. 1871. 486.
V. Über Lecithin und Nuclein in der Bierhefe. — Z. f. physiolog. Chemie. 1879. II. 427 ; III. 374.
VI. Pflüger's Archiv. 1876. XII. 7, 9.
VII. Physiolog. Chemie. 1877. I. 116. Archiv. Hyg. 1876. XII. 14.

VIII. Medic.-chem. Untersuchungen. 216.
IX. Ber. d. D. Chem. Ges. 1871. IV. 810.

Hoppe-Seyler, F., und Araki, Tr.,
I. Über die Einwirkung der bei Sauerstoffmangel im Harne ausgeschiedenen Milchsäuren auf polarisiertes Licht und die Rotationswerte aktiver Milchsäuren im Allgemeinen. — Z. f. physiolog. Chemie. 1895. XX. 365. — Ch. C. 1895. I. 703.

Horn, Franz Maximilian,
I. Benzoesäure in der Milch.—Zeitschrift für die chemische Industrie. 1887. 239. —Ch. C. 1888. 260.

Horváth, Alexis,
I. Über den Einfluss der Ruhe und der Bewegung auf das Leben. — Pflüger's Archiv für Physiologie. 1878. XVII. 125.

Hosaeus, Hans,
See Koch u. Hosaeus.

Hotter, Eduard,
I. Über den Gehalt an schwefeliger Säure der steirischen Obst- und Traubenweine. — IV. Jahresbericht d. Pomolog. Landes-Versuchs- und Samen-Kontroll-Station in Graz für 1895-96. 26.
II. Versuche und Untersuchungen über den Einfluss verschiedener Weinhefen auf die Vergärung des Apfelmostes.—I. Jahresber., etc. Graz. 1894. 25, 28.
III. Versuche über Beerenwein-Bereitung. — II. Jahresbericht, etc. Graz. 1894. 19.
See also Nobbe, Schmid, Hiltner u. Hotter.

Hotz,
I. Über Konservierung der Hefe.— American Brewer's Review. 1896. IX. 297.

Hoyer, D. P.,
I. Die Generationsdauer verschiedener Hefearten.—C. f. B. 2. Abt. 1899. V. 703.—K. J. X. 105.

Hubert, A.,
See Nivière et Hubert, Fernbach et Hubert.

Hüfner, G.,
I. Ausscheidung von freien Stickgas bei der Verwesung stickstoffhaltiger organischer Materie. — Journal f. prakt. Chemie. 1876. XIII. 292.—Biedermann's Centralblatt. 1877. XI. 74.—Ch C. 1876. 423.

Hueppe, Ferdinand,
I. Die Methoden der Bakterien-Forschung. 5th Ed. 1891. Wiesbaden. C. W. Kreidel.
II. Die Formen der Bakterien und ihre Beziehungen zu den Gattungen und Arten. Wiesbaden. 1886. Kreidel. 4*s*.
III. Über einige Vorfragen zur Desinfektionslehre und über die Hitze als Desinfektionsmittel.—Deutsche militärärztliche Zeitschrift. 1882. No. 2.
IV. Untersuchungen über die Zersetzungen der Milch durch Mikroorganismen.— Mitteilung. aus dem K. Gesundheits-Amte. 1884. II. 309.—Ch. C. 1884. 315.
V. Über die Verwendung von Eiern zu Kulturzwecken.—C. f. B. 1888. IV. 80.
VI. Naturwissenschaftliche Einführung in die Bakteriologie.—Wiesbaden. 1896. C. W. Kreidel.
VII. Uber Milchsterilisierung und über bittere Milch mit besonderer Rücksicht auf die Kinder-Ernährung. — Berliner klinische Wochenschrift. 1891. 717.— Ch. C. 1891. II. 722.—K. J. II. 191.
VIII. Über Chlorophyllwirkung chlorophyllfreier Pflanzen.—Tageblatt der 60. Naturforscherversammlung zu Wiesbaden. 1887. 244.—C. f. B. 1888. III. 420.—Ch. C. 1887. 1512.

Hulle, L. van den, et Laer, H. van,
I. Nouvelles recherches sur les bières bruxelloises à fermentation dite spontanée.—Mém. cour. et autres mém. publ. par l'Acad. Royale de Belgique. 1891. XV.—Z. g. Br. 1891. XIV. 578. —W. f. Br. 1891. VIII. 952.—K. J. II. 131.
II. Mém. cour., etc., par l'Acad. Royale de Belgique. 1890.—Abst. in W. f. Br. 1891. VIII. 984.

Huppert, H.,
I. Anleitung zur qualitativen und quantitativen Analyse des Harns. 10th Ed. Wiesbaden. 1898.

Husemann, August u. Theodor,
I. Die Pflanzenstoffe in chemischer, physiologischer, pharmakologischer und toxikologischer Hinsicht. 2nd Ed. 1882.

Husemann, Th.,
I. Über Brot.—Pharmaceutische Zeitung. Berlin. 1892. XXXVII. 128. — Ch. C. 1892. I. 561.
II. Notices from the Pharmaceut. Zeitschrift f. Russland, 1866, 572, in Jahresbericht ü. d. Fortschritte d. Pharmakognosie, Pharmacie und Toxikologie. 1866. I. 428.

Huth, S. von,
I. Wie man im Brauereibetriebe der Sarcina Einhalt thut. — Allgem. Zeitschrift f. Brauerei und Malzfabrikation. 1888. 510.—Vierteljahrsschrift f. Nahrungsmittel-Untersuchung und Hygiene. 1888. III. 158.—Ch. C. 1888. 1539.

Hyde, Ch. F.,
I. On Fermentation. — Journal of the Federated Institutes of Brewing. 1895. I. 380.—Z. g. Br. 1896. XIX. 112.— W. f. Br. 1895. XII. 754.—K. J. VI. 141.
See also Miller and Hyde.

Ide, M.,
I. Anaérobiose du bacille commun de l'intestin et de quelques autres bactéries —La Cellule. 1891. VI. 325.—K. J. II. 244.

Ikeno,
I. Ber. d. D. Bot. Ges. 1903. XXI. 259.

Iljenko, Paul,
I. Über Fäulnisprodukte des Tierkaseins. —Annalen d. Chem. u. Pharm. 1847. LXIII. 264.

Iljinsky, N. W.,
I. Materialien zur Lehre über den Hospitalkwass.—Wratsch. 1881. 85. Russian.

Ilkewitsch,
I. Neue Methode zur Entdeckung von Tuberkelbacillen in der Milch mit der Centrifuge.—Münch. med. Wochenschr. 1892. No. 5.—C. f. B. 1892. XII. 441.—K. J. III. 177.

Immendorff, H.,
I. Beiträge zur Lösung der Stickstoff-Frage. — Landw. Jahrbücher. 1892. XXI. 281.—Ch. C. 1892. I. 394.—K. J. III. 217.
II. Die Wirksamkeit der wichtigsten chemischen Konservierungsmittel des Stalldüngers.—Journal f. Landw. 1893. XXXXI. 1.—Ch. C. 1893. II. 115.
III. Zur Frage der Stickstoffkonservierung im Stalldünger.—Journal f. Landw. 1894. XXXXII. 69.—Ch. C. 1894. I. 1113.—K. J. V. 261.
See also Tacke, Immendorff, Hessenland, Schütte und Minssen.

Inouye, M.,
I. Die Bereitung und chemische Zusammensetzung von Tofu.—College of Agriculture. Tokio. Bulletin. 1895. II. 208.—Ch. C. 1896. I. 127.
II. Bemerkungen über Nukamiso.—College of Agriculture. Tokio. Bulletin. 1895. II. 216.—Ch. C. 1896. I. 128.

Inui, T.,
I. Journ. College of Science, Univ. Tokyo. 1901. XV. 465.
II. *Ibid.* 1901. XV. 405.
III. W. f. Br. 1902. XIX. 218.

Irmisch, M.,
I. W. f. Br. 1889. VI. 201, 413.
II. *Ibid.* 1891. VIII. 1135.

Issaew,
I. Z. g. Br. 1900. XXIII. 796.

Istrati, C., und Proca, G.,
I. Zur Zusammensetzung der Braga.—Buletinul societătii de sciințe din Bucuresci. 1899. V. 78.—Ch. C. 1899. II. 317.

Istvánffi, Gyula von,
I. Über die Rolle der Zellkerne bei der Entwicklung der Pilze.—Ber. d. D. Bot. Ges. 1895. XIII. 452.—C. f. B. 2. Abt. 1896. II. 352.

Iwanoff, L.,
I. Beitr. z. chem. Physiologie u. Pathologie. 1902. I. 524.—C. f. B. 2. Abt. 1904. XIII. 139.
II. Z. f. physiolog. Chemie. 1904. XLII. 464.

Iwanowski, D.,
I. Über die Wirkung des Sauerstoffes auf die alkoholische Gärung.—Mélanges biologiques tirées du Bulletin de l'Acad. imp. des sciences de St. Pétersbourg. 1894. XIII.—K. J. V. 116.
II. C. f. B. 2. Abt. 1903. X. 151.
III. Bull. de l'Acad. imp. des sciences de St. Pétersbourg. 1893. Noticed in Koch's Jahresber. 1894. V. 116.

Iwanowski, D., u. Obrastzow, S.,
I. Über die Wirkung des Sauerstoffes auf die Gärung.—C. f. B. 2. Abt. 1901. VII. 305.—Ch. C. 1901. I. 1380.

Jaccard, M.,
See Chuard u. Jaccard.

Jacobsen, J. C.,
I. Über das Ausarten der Hefe und über Mittel zur Beseitigung derselben.—Allgem. Zeitschrift f. Bierbrauerei u. Malzfabrikation. 1884. XIII. 496.
II. Die Reinzüchtung von Brauereihefe.—Z. g. Br. 1885. VIII. 117.

Jacobsthal, H.,
I. Die Fettbildung bei der Reifung des Käses.—Pflüger's Archiv. 1893. LIV. 484.—Ch. C. 1893. II. 218.

Jacoby, C.,
I. Das Sphacelotoxin, der spezifisch wirksame Bestandteil des Mutterkornes. —Archiv f. experim. Pathologie und Pharmakologie. 1897. XXXIX. 85.—Ch. C. 1897. I. 1059.

Jacquemart, F.,
I. Les Ptomaïnes. Histoire et caractères. Mémoire couronné.—Journal de médecine, de chirurgie et de pharmacologie. Bruxelles. 1890. No. 18.—C. f. B. 1891. IX. 107.

Jacquemin, Georges,
I. Fabrication industrielle de l'acide lactique.—Bulletin de la Société chimique de Paris. 1891. (3.) V. 294.—Journal de Pharmacie et de Chimie. 1891. XXIII. 229.—Ch. C. 1891. I. 698.—K. J. II. 178.
II. Les Fermentations rationelles. Nancy. 1900. 12*s.* 6*d.*
III. A New or Improved Method of an Apparatus for the Manufacture of Pure Yeast.—Engl. Patent No. 21011, Sept. 22, 1896.—K. J. IX. 100.

Jago, W.,
I. Fermentation in its Relation to Bread-making.—The Journal of the Society of Chemical Industry. 1887. VI. 164.—Ch. C. 1887. 570.

Jaksch, Rudolf von,
I. Studien über den Harnstoffpilz.—Z. f. physiolog. Chemie. 1881. V. 395.
II. Über die Entwicklungsbedingungen des Micrococcus ureæ.—Medic. Centralblatt. 1880. XVIII. 180.—Ch. C. 1880. 214.
III. Ber. d. D. Chem. Ges. 1886. XIX. 782.

JALOWETZ, Eduard,
I. Über das Vorkommen der Glykase im Gersten- u. Mais-Darrmalz.—Mitteilgen. d. österr. Versuchsstation f. Brauerei u. Mälzerei. 1892. V. Heft.—K. J. III. 255.
II. Mitt. d. österr. Versuchsstation f. Brauerei u. Mälzerei in Wien. 1894.—Chem. Zeit. 1894. XVIII. 39.

JAMIESON, J.,
I. The Influence of Light on the Development of Bacteria. — Nature. 1882. XXVI. 244.

JANCZEWSKI, Eduard von,
I. Les périthèces du Cladosporium herbarum. — Anzeiger der Akademie d. Wissenschaften in Krakau. 1893. 271. —Bot. Ztg. 1894. LII. 71.
II. Cladosporium herbarum i jego najpospolitsze na zbożu towarzysze. — Rozpravy Akademii Umiejetności. 1895. Ser. II. Tom. VII. p. 143. With four plates. Also gives the antecedent bibliography.—Résumé in Anzeiger, etc. 1894. 187.—Bot. Ztg. 1894. LII. 359.—Zeitschr. f. Pflanzenkrankheiten. 1894. IV. 247, 325.
III. Polymorphisme du Cladosporium herbarum.—Anzeiger, etc., pro 1892. 417.

JANOWSKI, Th.,
I. Über den Bakteriengehalt des Schnees. —C. f. B. 1888. IV. 547.—Ch. C. 1889. I. 48.
II. Zur Biologie der Typhusbacillen.—C. f. B. 1890. VIII. 167.

JANSSENS, Fr. A.,
I. Beiträge zu der Frage über den Kern der Hefezelle.—C. f. B. 1893. XIII. 638.—Ch. C. 1893. II. 281.—K. J. IV. 46.

JANSSENS, Fr. A., et LEBLANC, A.,
I. Recherches cytologiques sur la cellule de levure.—La Cellule. 1898. XIV. 203.—Annales de micrographie. 1898. X. 113.—C. f. B. 2. Abt. 1898. IV. 930.

JANSSENS, F. A., et MERTENS, Ad.,
I. La Cellule. 1903. XX. 351.

JÉGON,
I. Beiträge zur Kenntnis mannithaltiger Weine und Bestimmung des Mannits. —Journal de Pharm. et de Chimie. 1893. XXVIII. 103.—Ch. C. 1893. II. 500. —K. J. IV. 152.

JEGUNOW, Michael,
I. Sur les sulfo-bactéries des limans d'Odessa.—Archives des Sciences biologiques de St. Pétersbourg. 1895. III. 381.—Ch. C. 1895. I. 1123.
II. Bakteriengesellschaften.—C. f. B. 2. Abt. 1896. II. 11.—K. J. VII. 58.
III. *Ibid.*—*Ibid.* 441.

JENSEN, C. O.,
I. Bakteriologiske Undersögelser over visse Mälke- og Smörfeil. 1891.—C. f. B. 1892. XI. 409.—K. J. II. 181.
II. C. f. B. 2. Abt. 1902. VIII. 251.
III. *Ibid.*, p. 11.

JENSEN, Orla,
I. Studien über die Lochbildung in den Emmenthaler Käsen.—C. f. B. 2. Abt. 1898. IV. 217.
See also FREUDENREICH u. JENSEN.

JENTYS, Stefan,
I. Über den Einfluss hoher Sauerstoffspannungen auf das Wachstum der Pflanzen. —Unters. a. d. Botan. Inst. zu Tübingen. 1888. II. 419.

JESERICH, P., und NIEMANN, F.,
I. Über einige Fälle von Wurst- und Fleischvergiftung. — Hygien. Rundschau. 1893. III. 813.—Ch. C. 1894. I. 210.

JESSER, J.,
I. Über Gärungsprodukte der Raffinose. Österr.-ung. Zeitschr. f. Zuckerindustrie u. Landwirtschaft. 1889. XVIII. 598. —Ch. C. 1890. I. 451.

JODLBAUER, Max,
I. Über die Anwendbarkeit der alkoholischen Gärung zur Zuckerbestimmung.—Zeitschrift f. Rübenzucker-Industrie. 1888.—Z. g. Br. 1888. XI. 252.—C. f. B. 1. Abt. 1888. IV. 168.
II. Z. g. Br. 1888. II. 252.
III. Z. d. Vereins f. Rübenzucker-Industrie. 1888. XXV. 308.

JOHAN-OLSEN, Olav,
I. Zur Pleomorphismusfrage.—C. f. B. 2. Abt. 1897. III. 273.
II. Die bei der Käsereifung wirksamen Pilze. I.—C. f. B. 2. Abt. 1898. IV. 161.—Ch. C. 1898. I. 952.
III. Medd. fra naturh. Forening in Kristiania. 1883. 50.

JOHANNSEN, W.,
I. Sur le gluten et sa présence dans le grain de blé.—Compte rendu des travaux du laboratoire de Carlsberg. 1884. II. 199.—German transl. in Z. g. Br. 1888. XI. 437.

JOHNE, A.,
I. Der Trichinenschauer. With 125 figs. 6th Ed. Berlin. 1898. Parey.—3*s.* 6*d.*, bound.

JOHNSON, E.,
I. English Patent No. 29183 of 1897.

JONES,
I. The Analyst. 1889. XIV. 81.

JÖNSSON,
I. Der richtende Einfluss strömenden Wassers auf wachsende Pflanzen und Pflanzenteile (Rheotropismus).—Ber. d. D. Bot. Ges. 1883. I. 512.

JÖNSSON, B.,
I. Entstehung schwefelhaltiger Ölkörper in den Mycelfäden von Penicillium glaucum.—Botan. Centralblatt. 1889. XXXVII. 201.—Has antecedent bibliography.

JÖRGENSEN, Alfred,
I. Sarcina.—Allgem. Brauer- u. Hopfenzeitung. 1890. 1569.—Z. g. Br. 1890. XIII. 458.

II. Der Ursprung der Weinhefen.—C. f. B. 2. Abt. 1895. I. 321.—Z. g. Br. 1895. XVIII. 155.—Ch. C. 1895. I. 1006. —K. J. VI. 38.

III. Über den Ursprung der Alkoholhefen. —Ber. d. gärungsphysiol. Lab. v. A. Jörgensen. I. 1895.—Z. g. Br. 1895. XVIII. 367.—C. f. B. 2. Abt. 1895. I. 823.—K. J. VI. 39.

IV. Über Pilze, welche Übergangsformen zwischen Schimmel und Saccharomyceshefe bilden und die in der Brauereiwürze auftreten.—C. f. B. 2. Abt. 1896. II. 41.—Ch. C. 1896. I. 1009.—K. J. VII. 35.

V. Die Hefenfrage.—C. f. B. 2. Abt. 1898. IV. 860.—K. J. IX. 35.

VI. Hansen's System of Pure Yeast Culture in English Top-Fermentation.—Transactions of the Institute of Brewing. 1894. VII. 227.—Z. g. Br. 1893. XVI. 299.—K. J. V. 184.

VII. Untersuchungen über das Ausarten der Brauereihefe.—Z. g. Br. 1898. XXI. 113.—C. f. B. 2. Abt. 1898. IV. 586.—K. J. IX. 91.

VIII. Zur Analyse der obergärigen Hefe in Brauereien und Brennereien nach Hansen's Methode.—Z. g. Br. 1891. XIV. 45.—C. f. B. 1. Abt. 1891. IX. 602.—K. J. II. 146.

IX. Die Aufbewahrung der ausgewählten Heferassen.—Allgem. Zeitschrift f. Bierbrauerei u. Malzfabrikation. 1890. XIX. 1215.—K. J. I. 70.

X. Die Behandlung der Würze mittelst der Centrifuge.—Z. g. Br. 1890. XIII. 49.

XI. Biological Studies on English Yeasttypes, with Particular Regard to their Practical Use.—Journal of the Federated Institutes of Brewing. 1899. V. 257. —K. J. X. 120.

XII. Die Mikroorg. d. Gärungs-industrie. 1898. 276.

XIII. *Ibid.* 1898.

Jörgensen, A., u. Bergh, A.,

I. Apparat zur kontinuierlichen Fortpflanzung von Mikroorganismen. German Patent No. 58075, Apr. 17, 1890.—W. f. Br. 1892. IX. 198.—K. J. III. 162.

Jost,

I. Vorlesungen u. Pflanzen-physiologie. Jena. 1904.

Joubert, J.,

See Pasteur et Joubert, *and* Pasteur, Joubert et Chamberland.

Jousset,

I. Comptes rendus de la Soc. de Biologie. Paris. 1903.—Abst. in Botan. Centralbl. 1904. XCV. 297.

Jubert, P.,

I. Über das Gummi der Rübensäfte.—Sucrerie indigène. 1874. No. 9 et 10. —Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1875. 105, 110. 1876. 747.

Juhler, John J.,

I. Umbildung eines Aspergillus in einen Saccharomyceten.—C. f. B. 2. Abt. 1895. I. 16, 326.—K. J. VI. 37.

Jumelle, Henri,

I. Sur une espèce nouvelle de bactérie chromogène, le Spirillum luteum.—C. R. 1892. CXV. 843.—C. f. B. 1893. XIII. 340.—K. J. III. 44.

Kabrhel, Gustav,

I. Über das Ferment der Milchsäuregärung in der Milch.—Allgem. Wiener mediz. Zeitung. 1889. No. 52 u. 53.—C. f. B. 1890. VII. 506.

II. Zur Frage der Stellung des Kaseins bei der Milchsäuregärung.—Archiv f. Hygiene. 1895. XXII. 392.—Ch. C. 1895. I. 1008.—C. f. B. 2. Abt. 1895. I. 439.

Kabsch, W.,

I. Untersuchungen über die chemische Beschaffenheit der Pflanzengewebe.—Jahrbücher f. wissenschaftl. Botanik. 1863. III. 357.

Kaiser, A.,

I. Chemische Untersuchung des Agaricus muscarius L.—Göttinger Dissert. 1862.

Kalanthar,

See Kalanthariantz.

Kalanthariantz, Anuschawan,

I. Über die Spaltung von Polysacchariden durch verschiedene Hefenenzyme. Dissert. Berlin. 1898.—Abstract in Z. f. physiolog. Chemie. 1898-99. XXVI. 88.—Ch. C. 1898. II. 1179.—C. f. B. 2. Abt. 1899. V. 43.

Kalt, H. P.,

I. Über die Bestimmung von ungelösten stickstoffhaltigen Verbindungen in der Maische.—Z. g. Br. 1886. IX. 489.

Kanter,

I. Über die Wirkung einiger Salze d. Schwermetalle auf d. Wachsthum u. chemische Zusammensetzung v. Asp. niger. Dissn. (Russian). 1903.

Kappes, Heinrich Constantin,

I. Analyse der Massenkulturen einiger Spaltpilze und der Soorhefe. Diss. 1890. Leipzig.—K. J. I. 28.

Karlinsky, Justyn,

I. Zur Kenntnis der Bakterien der Thermalquellen.—Hygien. Rundschau. 1895. V. 685.—Ch. C. 1895. II. 873.

II. Zur Kenntnis des Bacillus enteritidis Gärtner.—C. f. B. 1889. VI. 289.

Kassner, G.,

I. Apothekerzeitg. 1869. XI. 584.

Katz, Julius,

I. Die regulatorische Bildung von Diastase durch Pilze.—Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1898. XXXI. 599.—C. f. B. 2. Abt. 1899. V. 288.—Z. g. Br. 1898. XXI. 434.—K. J. IX. 277.

KATZ, Oskar,
I. Zur Kenntnis der Leuchtbakterien.—C. f. B. 1891. IX. 157. Has the bibliography for 1887 to 1891.—K. J. II. 261.
II. Jahr. wiss. Bot. 1898. XXXIII. 599.

KAYSER, Edmund,
I. Etudes sur la fermentation lactique.—Ann. Inst. Past. 1894. VIII. 737.—C. f. B. 2. Abt. 1895. I. 436.—Ch. C. 1895. I. 92.—K. J. V. 235.
II. Les levures. Paris. 1896.
III. Etudes sur la fermentation du cidre.—Ann. Inst. Past. 1890. IV. 321.—C. f. B. 1890. VIII. 726.—Ch. C. 1891. I. 385.—K. J. I. 61.
IV. Contribution à l'étude physiologique des levures alcooliques du lactose.—Ann. Inst. Past. 1891. V. 395.—C. f. B. 1. Abt. 1891. X. 418.—K. J. II. 138.
V. Note sur les ferments de l'ananas.—Ann. Inst. Past. 1891. V. 456.—C. f. B. 1. Abt. 1891. X. 489.—K. J. II. 134.
VI. Über die Lebensdauer der Hefen.—La Bière. 1895, p. 14.—K. J. VI. 145.
VII. Contributions à l'étude des levures de vin.—Ann. Inst. Past. 1892. VI. 569.—C. f. B. I. Abt. 1893. XIII. 389.—Ch. C. 1893. I. 838.—K. J. III. 114.
VIII. Action de la chaleur sur les levures.—Ann. Inst. Past. 1889. III. 513.—C. f. B. 1. Abt. 1890. VII. 201.
IX. Ann. Inst. Past. 1896. X. 51.
X. *Ibid.* 1900. XIV. 605.
XI. Le Cidre. 1903. XX. 351.
XII. *Ibid.* 1890. III. 371.
XIII. Ann. Inst. Past. 1893. VII. 41.

KAYSER, Ed., et BARBA, G.,
I. Contribution à l'étude des levures de vin.—Bulletin du Ministère de l'Agriculture. Paris. 1896.—K. J. VII. 126.

KAYSER, Edm., und BOULLANGER, E.,
I. Etude sur les ferments naturels de l'hydromel. Paris. 1897.—Z. g. Br. 1897. XX. 584.—K. J. VIII. 136.
II. Studien über die Bildung des Glycogens in der Hefe.—Annales de la Brasserie et de la Distillerie. 1898.—Ch. C. 1898. II. 440.—K. J. IX. 75.

KAYSER u. DIENERT,
I. Bull. de l'Assoc. d. Chimistes. 1901. XIX. 353.

KAYSER, R.,
I. Über den Gehalt der Weine an schwefliger Säure.—Zeitschr. f. öffentl. Chemie. 1897. III. 513.—Ch. C. 1897. II. 1121.

KEDROWSKI, W.,
I. Über zwei Buttersäure produzierende Bakterien-Arten.—Z. f. Hygiene. 1894. XVI. 444.—C. f. B. 1894. XVI. 124.—Ch. C. 1894. II. 48.—K. J. V. 266.

KELLER, C. C.,
I. Mitteilungen über die Wertbestimmung von Drogen.—Schweizer Wochenschrift f. Pharmacie. 1894. XXXII. 121, 133. 1896. No. 8.—Ch. C. 1894. I. 931, 932. 1896. I. 765.

KELLNER, MORI, u. NAGOAKA,
I. Z. f. physiolog. Chemie. 1889. XIV. 293.

KELLNER, O.,
I. Chem. Ztg. 1895. XIX. 120, 265.

KELLNER, Oskar,
I. Bildung von Fett aus Eiweisskörpern beim Reifen des Käses.—D. Landw. Versuchs-Stationen. 1880. XXV. 39.
II. Über die Bereitung von Saké, Shoyou und Miso.—Chemiker-Zeitung. 1895. XIX. 97.—Ch. C. 1895. I. 454.
III. Über das Verhalten des Harnstoffs im Ackerboden.—Landw. Jahrbücher. 1886. XV. 712.—C. f. B. 1887. I. 40.

KELLNER, O., und YOSHII, T.,
I. Über die Entbindung freien Stickstoffs bei der Fäulnis und Nitrifikation.—Z. f. physiolog. Chemie. 1887. XII. 95.—Biedermann's Centralblatt. 1887. XXI. 854.—Ch. C. 1887. 1552.—C. f. B. 1888. III. 301.

KERN, Eduard,
I. Über ein neues Milchferment aus dem Kaukasus.—Bulletin de la Société Impériale des Naturalistes de Moscou. 1881. No. 3.—Bot. Ztg. 1882. XL. 264.—Biolog. Centralbl. 1882. II. 137.

KERRY, R., und FRAENKEL, S.,
I. Über die Einwirkung der Bazillen des malignen Ödems auf Kohlehydrate.—Monatshefte f. Chemie. 1890. XI. 268. 1891. XII. 350.—K. J. I. 141; II. 239.
See also OBERMAYER und KERRY.

KHONDABACHIAN,
I. Ann. Inst. Past. 1892. VI. 600.

KIESENWALTER, A.,
I. Die Konservierung der Hefe.—Norddeutsche Brauerzeitung. 1886. 335.

KILIANI, H., und BAZLEN, M.,
I. Ber. d. D. Chem. Ges. 1894. XXVII. 3115.

KIRCHNER, Oskar,
I. Die Krankheiten und Beschädigungen unserer landw. Kulturpflanzen. Stuttgart. 1890. E. Ulmer. 9*s.*
II. Die Wurzelknöllchen der Sojabohne.—Cohn's Beiträge, etc. 1894. VII. 213.

KIRCHNER, O., u. BOLTSHAUSEN, H.,
I. Atlas der Krankheiten und Beschädigungen unserer landwirtschaftl. Kulturpflanzen. Stuttgart. E. Ulmer. 1896.—I. Reihe: Getreide.—II. R.: Hülsenfrüchte, Futtergräser u. Futterkräuter.—III. R.: Wurzelgewächse und Handelsgewächse.—IV. R.: Gemüse- und Küchenpflanzen.—V. R.: Obstbäume.—VI. R.: Weinstock und Beerenobst.

KISSLING, E.,
I. Zur Biologie der Botrytis cinerea.—Dissert. Bern. 1889.—C. f. B. 1890. VII. 543.

Kitasato, Shibasaburo, u. Weyl, Th.,
I. Zur Kenntnis der Anaëroben.—Z. f. Hygiene. 1890. VIII. 41. 1891. IX. 97.—C. f. B. 1890. VIII. 12.—K. J. I. 14; II. 73.

Kitics Án, P.,
I. Ber. d. D. Chem. Ges. 1883. XVI. 1179.

Kjeldahl,
I. Medd. f. Carlsberg. Labor. 1881. III. 186.

Kladakis, Th. M.,
I. Über die Einwirkung des Leuchtgases auf die Lebenstätigkeit der Mikroorganismen. Dissert. Berlin. 1890.—C. f. B. 1890. VIII. 23.

Klarverweiden, Th. J.,
I. Eenige opmerkingen omtrent Blauwe Kaas. — Amsterdam. 1894. J. J. de Bussry.—Germ. transl. in Milchzeitung. 1894. 540.—Ch. C. 1894. II. 712.

Klason,
I. Z. g. Br. 1898. XXI. 632.

Klaudi, Josef, und Svoboda, Anton,
I. Analyse von Flaschenbier. — Listy chemické. 1890.—Allgem. Brauer- und Hopfenzeitung. 1890. 2079.—K. J. I. 66.

Klebs, Georg,
I. Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896. G. Fischer. 18*s*.—Bot. Ztg. 2. Abt. 1897. LV. 38.
II. Zur Physiologie der Fortpflanzung einiger Pilze.—Jahrbücher f. wissenschaftl. Botanik. 1898. XXXII. 1.—Bot. Ztg. 2. Abt. 1898. LVI. 278.

Klecki, Valerian von,
I. Ein neuer Buttersäure-Gärungserreger (Bacillus saccharobutyricus) und dessen Beziehungen zur Reifung und Lochung des Quargelkäses.—C. f. B. 2. Abt. 1896. II. 169.—Ch. C. 1896. II. 253.—K. J. VII. 189.

Klein, Carl,
I. Beitrag zur Kenntnis des roten Malzschimmels.—Mitteilungen der Österr. Versuchsstation f. Brauerei u. Mälzerei in Wien. 1892. V.—C. f. B. 1. Abt. 1893. XIII. 196.

Klein, E.,
I. Über ein neues Milchferment aus dem Kaukasus.—Bulletin de la Soc. imp. des naturalistes de Moscou. 1881, p. 141.

Klein, E., u. Gordon, M.,
I. C. f. B. 1. Abt. 1903. XXXV. 138.

Klein, Ludwig,
I. Botanische Bakterienstudien. I.—C. f. B. 1889. VI. 313.
II. Über einen neuen Typus der Sporenbildung bei den endosporen Bakterien.—Ber. d. D. Bot. Ges. 1889. VII. 57.
III. Über die Ursachen der ausschliesslich nächtlichen Sporenbildung bei Botrytis cinerea.—Bot. Ztg. 1885. XXXXIII. 6.

Kleinschmidt, K., jr.,
I. German Patent No. 105573, Nov. 26, 1898.—Ch. C. 1900. I. 160.

Klöcker, Albert,
I. Recherches sur le Saccharomyces Marxianus, Sacch. apiculatus et Sacch. anomalus.—Compte rendu des travaux du laborat. de Carlsberg. 1895. IV. 20.—Annales de Micrographie. 1895. VII. 313.—C. f. B. 2. Abt. 1895. I. 446.—K. J. VI. 34.
II. Die Gärungsorganismen in der Theorie und Praxis der Alkoholgärungsgewerbe. Stuttgart. 1900. 8*s*.
III. Comptes rendus, etc., de Carlsberg. 1895. IV. 22.
IV. *Ibid.* 1900. V. 58, 59.
V. C. f. B. 2. Abt. 1902. VIII. 129.—Comptes rendus, etc., de Carlsberg. 1903. VI. 84.
VI. Comptes rendus, etc., de Carlsberg. 1903. VI. 92. C. f. B. 2. Abt. 1904. XII. 501.
VII. Die Gärungsorganismen. Stuttgart. 1900, p. 242.

Klöcker, Alb., und Schiönning, H.,
I. Experimentelle Untersuchungen über die vermeintliche Umbildung des Aspergillus oryzæ in einen Saccharomyceten.—C. f. B. 2. Abt. 1895. I. 777.—K. J. VI. 40.
II. Experimentelle Untersuchungen über die vermeintliche Umbildung verschiedener Schimmelpilze in Saccharomyceten.—C. f. B. 2. Abt. 1896. II. 185.—K. J. VII. 33.
III. Que savons-nous de l'origine des Saccharomyces?—Compte rendu des travaux du Laboratoire de Carlsberg. 1896. III. 36.—Annales de Micrographie. 1897. IX. 233.—C. f. B. 2. Abt. 1897. III. 193.—K. J. VII. 34.
IV. Noch einmal Saccharomyces und Schimmelpilze.—C. f. B. 2. Abt. 1898. IV. 460.
V. Über Durchwachsungen und abnorme Konidienbildungen bei Dematium pullulans de Bary und bei anderen Pilzen.—C. f. B. 2. Abt. 1899. V. 505. 1901. VII. 152.
VI. Medd. fra Carlsberg Labor. 1896. IV. 120.
VII. Comptes rendus, etc., de Carlsberg. 1900. V. 47.
VIII. *Ibid.* 1896. IV. 36.

Knecht, W.,
I. C. f. B. 2. Abt. 1901. VII. 161.

Knieriem, Woldemar von, u. Mayer, Adolf,
I. Über die Ursache der Essiggärung.—D. Landw. Versuchsstationen. 1873. XVI. 305.—Ch. C. 1873. 666.

Knoesel, C.,
I. C. f. B. 2. Abt. 1902. VIII. 241.

Kny, L.,
I. Die Beziehungen des Lichtes zur Zellteilung bei Saccharomyces cerevisiæ.—Ber. d. D. Bot. Ges. 1884. II. 129.—Reproduced in Z. g. Br. 1884. VII. 210.
II. Unterrichtsblatt Math. u. Naturw. 1902. No. 1.

Kobert, Rudolf,
I. Über die Bestandteile und Wirkungen des Mutterkorns.—Archiv für experim. Pathologie. 1884. XVIII. 316.—Ch. C. 1885. 66. 1895. II. 175.
II. Über den Kwass und dessen Bereitung. —Histor. Studien a. d. pharmakolog. Inst. d. K. Universität Dorpat. 1896. V.—C. f. B. 2. Abt. 1897. III. 253.—Therapeut. Monatshefte. 1895. IX. 273.—Ch. C. 1895. II. 240.—K. J. VI. 155; VII. 119.
III. Lehrbuch d. Intoxicationen. 1904. II. 186.
IV. Pflüger's Archiv. 1903. XCIX. 116.

Koch, Alfred,
I. Über Morphologie und Entwicklungsgeschichte einiger endosporer Bakterienformen. Habilitationsschrift. Göttingen.—Bot. Ztg. 1888. XXXXVI. 277. —C. f. B. 1888. IV. 358.
II. Apparat zum Filtrieren bakterienhaltiger Flüssigkeiten.—Zeitschrift f. wissenschaftl. Mikroskopie. 1891. VIII. 186.—K. J. II. 21.
III. Vergleichende bakteriologische Untersuchung über die Haltbarkeit der Norweger und Nordsee-Schellfische.—Mitteilungen der Sektion für Küsten-und Hochsee-Fischerei. 1894. No. 8.—C. f. B. 1894. XVI. 967.
IV. Zur Kenntnis der Fäden in den Wurzelknöllchen der Leguminosen. — Bot. Ztg. 1890. XXXXVIII. 607.—C. f. B. 1890. VIII. 709.—K. J. I. 129.
V. Weinbau u. Weinhandel. 1898, p. 236.

Koch, Alfred, u. Hosaeus, Hans,
I. Über einen neuen Froschlaich der Zuckerfabriken.—C. f. B. 1894. XVI. 225.—Ch. C. 1894. II. 703.—K. J. V. 58.
II. Das Verhalten der Hefen gegen Glycogen.—C. f. B. 1894. XVI. 145.—Ch. C. 1894. II. 869.—K. J. V. 110.

Koch, Friedrich,
I. Experimentelle Prüfung des Holzgummi und dessen Verbreitung im Pflanzenreiche.—Pharmac. Zeitschrift f. Russland. 1886. XXV. 619.—Ber. d. D. Chem. Ges. 1887. XX. Ref. 145.

Koch, Robert,
I. Über Desinfektion.—Mitteilungen a. d. Kaiserl. Gesundheits-Amte. 1881. I. 252.—Ch. C. 1882. 509.

Koch, R., und Wolffhügel, G.,
I. Untersuchungen über Desinfektion mit heisser Luft.

Kochs, W.,
I. Gibt es ein Zellleben ohne Mikroorganismen ?—Biolog. Centralblatt. 1894. XIV. 481.—Botan. Centralblatt. 1895. LXII. 112.

Koernicke,
I. Ber. d. D. Bot. Ges. 1904. XXII. 163.

Kogelmann, Franz,
I. Über Milchwein (Kefir). — Deutsche Med. Ztg. 1886.—Pharmaceut. Centralhalle. 1886. XXVII. 42.

Kohl, F. G.,
I. Über den Polymorphismus von Pleospora herbarum Tulasne.—Botan. Centralblatt. 1883. XVI. 26.

Köhler, Julius,
I. Saccharomyces membranæfaciens Hansen.—Mitteilungen d. Österr. Versuchs-Station f. Brauerei, etc. 1892. V.—C. f. B. 1. Abt. 1893. XIII. 131.—K. J. III. 140.

Kohlrausch, Otto,
I. Über die Zusammensetzung einiger essbaren Pilze mit besonderer Berücksichtigung ihres Nahrungswertes. Dissert. Göttingen. 1867.

Kohnstamm, P.,
I. Bot. Centralbl. 1901. X. Beiheft 2, p. 91.

Kohnstein, B.,
I. Beitrag zur Kenntnis der säurebildenden Stoffe in den Gerberbrühen.—Der Gerber. 1886. No. 293.

Kokosinski, Ed.,
I. Application industrielle de la méthode Hansen à la fermentation haute (dans le Nord de la France).—Comptes rendus de la Société des brasseries belges. Station scientifique de Brasserie. Gand. 1890. I. 13.—Z. g. Br. 1891. XIV. 56.—K. J. I. 70.

Kolbe,
I. Liebig's Ann. 1860. CXIII. 244.

Kolkwitz, R.,
I. Über den Einfluss des Lichtes auf die Atmung der niederen Pilze.—Pringsheim's Jahrbücher f. w. Bot. 1898. XXXIII. 128.—C. f. B. 2. Abt. 1899. V. 222.—Ch. C. 1899. I. 1250.

Komeros, K., u. Haunalter, E. von,
I. Z. f. d. landw. Versuchswesen in Österreich. 1902. V. 1225.

König, J.,
I. Chemie der menschlichen Nahrungs- und Genussmittel. 3rd Ed. Berlin. 1889–1893.

Korff, Gustav,
I. Einfluss des Sauerstoffs auf Gärung, Gärungsenergie und Vermehrungsvermögen verschiedener Heferassen unter verschiedenen Ernährungsbedingungen. Dissert. Erlangen. 1898.—C. f. B. 2. Abt. 1898. IV. 465.—Ch. C. 1898. II. 441.—K. J. IX. 79.

Korschelt, O.,
I. Über Sake, das alkoholische Getränk der Japaner. — Dingler's polytechn. Journal. 1878. CCXXX. 76.
II. Mitteil. d. D. Ges. f. Natur- und Völkerkunde Ostasiens. 1876. Heft 16. 240.
Kosai, J., und Yabe, K.,
I. Über die bei der Sakebereitung beteiligten Pilze.—C. f. B. 2. Abt. 1895. I. 619.—K. J. VI. 152.
Kosinski,
I. Jahr. wiss. Bot. 1901. XXXVII. 137.
Kosjatschenskow,
I. Journ. f. experim. Landw. 1903. 439. (Russian.) — Bot. Centralbl. 1904. XCV. 591.
Kossel, Albrecht,
I. Zur Chemie des Zellkerns.—Z. f. physiolog. Chemie. 1883. VII. 7.
II. Über die chemische Zusammensetzung der Zelle.—Archiv d. Anatomie u. Physiologie. 1891. Physiolog. Abt. 181.—Ch. C. 1891. II. 37.
III. Über das Nuclein der Hefe.—Z. f. physiol. Chemie. 1879. III. 284. 1880. IV. 290.—Ch. C. 1879. 583.
IV. Über die Nucleine.—Centralbl. f. die mediz. Wissenschaften. 1889. XXVII. 417, 593.—Ch. C. 1889. II. 143, 504.
V. Über die Nucleinsäure.—Archiv d. Anatomie und Physiologie. Physiolog. Abt. 1893. 157.—Ch. C. 1893. I. 787.
VI. Nucleinsäure.—Centralbl. f. d. mediz. Wissenschaften. 1893. 497.—Ch. C. 1893. II. 649.
VII. Über die Herkunft des Hypoxanthins in den Organismen.—Z. f. physiolog. Chemie. 1881. V. 152.
VIII. Über die Verbreitung des Hypoxanthins im Tier- und Pflanzenreich. *Ibid.* 1881. V. 267.
IX. Über Xanthin und Hypoxanthin.—*Ibid.* 1882. VI. 422.
X. Über Guanin.—*Ibid.* 1884. VIII. 404.
XI. Weitere Beiträge zur Chemie des Zellkernes.—*Ibid.* 1886. X. 248.—Ber. d. D. Chem. Ges. 1885. XVIII. 79, 1928.
Kossel, A., und Dakin, H. D.,
I. Z. f. physiolog. Chemie. 1904. XLI. 321; XLII. 181.
Kossel, A., und Neumann, A.,
I. Über das Thymin, ein Spaltungsprodukt der Nucleinsäure.—Ber. d. D. Chem. Ges. 1893. XXVI. 2753.—Ch. C. 1894. I. 40.
II. Darstellung und Spaltungsprodukte der Nucleinsäure (Adenylsäure).—Ber. d. D. Chem. Ges. 1894. XXVII. 2215.—Ch. C. 1894. I. 1158; II. 708.
Kossowicz, Alexander,
I. Untersuchungen über das Verhalten der Hefen in mineralischen Nährlösungen. I.—Z. f. d. landw. Versuchswesen in Österreich. 1903. VI. 27.—Ch. C. 1903. I. 475.
II. Z. f. d. landw. Versuchswesen in Österreich. 1903. VI. 731.
III. *Ibid.* 1906. IX. 688.
Kossowitsch, P.,
I. Durch welche Organe nehmen die Leguminosen den freien Stickstoff au?—Bot. Ztg. 1892. L. 697.—Ch. C. 1893. I. 613.—K. J. III. 193.
II. Fixieren die Algen freien Stickstoff?—Bot. Ztg. 1. Abt. 1894. LII. 97.—Ch. C. 1894. II. 244.—K. J. V. 257.
Kostytschew,
I. Ber. d. D. Bot. Ges. 1902. XX. 327. 1904. XXII. 207.
Kosutány,
I. Landw. Versuchs-Stationen. 1898. XLIX. 174.
II. *Ibid.* 1892. XL. 217.
Kotljar, E.,
I. Zur Frage über den Einfluss des Lichtes auf Bakterien.—Wratsch. 1892. No. 39–40.—C. f. B. 1892. XII. 836.—K. J. III. 77.
Kozai, Y.,
I. Chemische und biologische Untersuchungen über Sake-Bereitung.—C. f. B. 2. Abt. 1900. VI. 385.—Ch. C. 1900. II. 220.
II. C. f. B. 2. Abt. 1900. VI. 385.
Krafkoff,
I. Zur Frage vom Glycogen der Pilze.—Scripta botanica horti universitatis Imperialis Petropolitanæ. III. fasc. I. p. 17.
Král, Franz,
I. Weitere Vorschläge und Anleitungen zur Anlegung von bakteriologischen Museen.—Z. f. Hygiene. 1889. V. Heft 3.—C. f. B. 1889. V. 392.
See also Soyka und Král.
Kramer, Ernst,
I. Bakteriologische Untersuchungen über das Umschlagen des Weines.—D. landw. Vers.-Stat. 1890. XXXVII. 325.—C. f. B. 1891. IX. 268.—Ch. C. 1890. II. 180.—K. J. I. 141.
II. Studien über die schleimige Gärung.—Sitzungsber. d. k. Akad. d. Wiss. in Wien. Monatshefte für Chemie. 1889. X. 467.—C. f. B. 1890. VIII. 77.
III. Österr. landw. Centralbl. 1891. I. 30.—Abst. in Chem. Centralbl. 1891. II. 707.
Krämer, G., und Pinner, A.,
I. Über die Destillationsprodukte des Rohspiritus.—Ber. d. D. Chem. Ges. 1869. II. 401. 1870. III. 75.
Krannhals, H.,
I. Über das kumysähnliche Getränk Kefir und über den Kefir-Pilz.—Deutsches Archiv f. klin. Medicin. 1884. XXXV. 18.
Krasnosselsky, T.,
I. C. f. B. 2. Abt. 1904. XIII. 673.
II. Ber. d. D. Bot. Ges. 1905. XXIII. 142.

KRASSER, Fridolin,
I. Über das angebliche Vorkommen eines Zellkerns in den Hefezellen. — Österr. Bot. Zeitschrift. 1885. XXXV. 373.—Bot. Ztg. 1886. XXXXIV. 389.
II. Über den Zellkern der Hefe.—Österr. Bot. Zeitschrift. 1893. XLIII. 14.—Bot. Ztg. 1893. LI. 49.—K. J. IV. 45.

KRATSCHMER, Florian, und NIEMILOWICZ,
I. Über eine eigentümliche Brotkrankheit. —Wiener klinische Wochenschrift. 1889. No. 30.—C. f. B. 1889. VI. 501.—Ch. C. 1889. II. 981.

KRAUCH, C.,
I. Beiträge zur Kenntnis der ungeformten Fermente im Pflanzenreich.—D. landw. Vers.-Stat. 1879. XXIII. 77.
II. Über peptonbildende Fermente in den Pflanzen.—*Ibid.* 1882. XXVII. 383.

KRAUS,
I. Versuche mit Pflanzen im farbigen Licht.—Sitzgsber. d. Naturforschenden Gesellschaft in Halle. 1876.—Bot. Ztg. 1876. XXXIV. 505.

KRESSEL, Edward,
I. German Patent No. 89819 of 1896.
See also HILL-JONES und KRESSEL.

KRIEGER,
I. Über die Veränderungen, welche die Bestandteile der Lagerbiere während der Lagerung erleiden. — Amerikan. Bierbrauer. 1894.—K. J. V. 141.

KRÖBER, E.,
I. Z. g. Br. 1895. XVIII. 337.

KRÖNIG, Bernhard, und PAUL, Theodor,
I. Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfektion. — Z. f. Hygiene. 1897. XXV. 1. A bibliography of the subject is given.—Ch. C. 1897. II. 369.—K. J. VIII. 54.
See also PAUL und KRÖNIG.

KRÜCKMANN, Emil,
I. Eine Methode zur Herstellung bakteriologischer Museen und Konservierung von Bakterien.—C. f. B. 1894. XV. 851.—K. J. V. 30.

KRUEGER, R.,
I. Bakteriologisch-chemische Untersuchungen käsiger Butter.—C. f. B. 1890. VII. 425.—K. J. I. 87.
II. Über bittere Milch.—Molkereizeitung. 1890. No. 30.
III. Molkereizeitung. Hildesheim. 1892. Nos. 34–38.

KRUEGER, S.,
I. Über den Einfluss des konstanten elektrischen Stromes auf Wachstum und Virulenz der Bakterien.—Zeitschrift für klinische Medizin. 1893. XXII. 190. —Ch. C. 1893. I. 484.—K. J. IV. 115.

KRÜGER, Friedrich,
I. Über den Einfluss von Kupfervitriol auf die Vergärung von Traubenmost durch Saccharomyces ellipsoideus.—C. f. B. 2. Abt. 1895. I. 10.—Ch. C. 1895. I. 696.

KRÜGER, Wilhelm,
I. Über zwei aus Saftflüssen reingezüchtete Algen.—Zopf's Beiträge zur Physiologie und Morphologie niederer Organismen. 4. Heft. 91.—Leipzig. 1894. Engelmann.

KRUIS, K., und RAÝMAN, B.,
I. Etudes chimique et biologique. IIe partie.— Bulletin de l'Académie des sciences de l'Empereur François Joseph I. Classe des sciences mathematiques et naturelles. Prag. 1894. I.—Z. g. Br. 1895. XVIII. 190.—C. f. B. 2. Abt. 1895. I. 637.—Ch. C. 1895. II. 311.—K. J. V. 143.
II. Z. f. Spiritusind. 1896. XIX. 131.
III. *Ibid.* 1904. XXVII. 311.
See also RAÝMAN und KRUIS.

KRUTZSCH,
I. J. f. pr. Chem. 1844. XXXI. 1.
II. Liebig's Ann. 1844. LII. 311.

KRYSIŃSKI, S.,
I. Pathologische und kritische Beiträge zur Mutterkornfrage. Jena. 1888. G. Fischer.—Has the antecedent bibliography relating to toxicology.

KUBAREW, G. W., u. TERESCHTSCHENKO, N. A.,
I Über Soldatenkwass.—Wratsch. 1897. No. 47.—Pharm. Z. f. Russland. 1897. XXXVI. 733.—Ch. C. 1898. I. 644.

KÜHN, Julius,
I. Das Einsäuern (Einmachen) der Futtermittel. Reprinted from Mentzel u. v. Lengerke's landwirt. Kalender für 1885. II. Teil. Berlin. Parey.

KÜHN, M.,
I. Versuche über Konservierung der Milch für analytische Zwecke.—Der Landwirt. 1894. XXX. 239.—Ch. C. 1894. II. 460.

KÜHNE, W.,
I*a*. Lehrbuch der physiologischen Chemie. 1868. 334.

KÜHNE, W.,
I. Kieselsäure als Nährboden für Organismen.—Z. f. Biologie. 1890. XXVII. 172.—C. f. B. VIII. 410.—K. J. I. 11.

KÜSTER, E.,
I. Zur Kenntnis der Bierhefe.—Biolog. Centralblatt. 1898. XVIII. 305.—C. f. B. 2. Abt. 1899. V. 196.

KÜTZING, Friedrich Traugott,
I. Mikroskopische Untersuchungen über die Hefe und Essigmutter.—Journal f. prakt. Chemie. 1837. XI. 385.
II. Sphærotilus natans.—Linnea. 1833. VIII. 385.
III. Phycologia generalis. 1843.
IV. Species algarum. 1849. 145.
V. Phycologia generalis. 1843, p. 148.

KUKLA, Anton,
I. Die Reinhefe in Böhmen.—Ber. d. Versuchsanstalt für Brauindustrie in Böhmen für 1890.—K. J. II. 147.

Kulisch, Paul,
I. Über die Abnahme der Säure in Obst und Traubenweinen während der Gärung und Lagerung.—Weinbau und Weinhandel. 1889. Nos. 42-44.—Landw. Jahrbücher. 1890. XIX. 83.—Chemikerzeitung. 1889. XIII. 1407.—Ch. C. 1889. II. 954.
II. Die deutschen Ausleseweine, ihr Werden und Wesen. Vortrag.—Weinbau und Weinhandel. 1895. XIII. 389.—Ch. C. 1896. I. 72.—K. J. VI. 149.
III. Untersuchungen über das Böcksern der Weine.—Weinbau und Weinhandel. 1895. XIII. 2.—K. J. VI. 147.
IV. Z. f. angew. Chem. 1896. 418.

Kupfer u. Voit,
I. Münch. med. Wochenschrift. 1897. XLIV. 321.

Kuprianow, J.,
I. Beiträge zur Biologie der Vibrionen.—A. f. Hygiene. 1893. XIX. 282, 291.—C. f. B. 1894. XV. 489.—Ch. C. 1894. I. 161, 280.—K. J. IV. 84.

Kurzwelly,
I. Jahr. wiss. Bot. 1900. XXXVIII. 291.

Kusserow, Reinhold,
I. Die Bedeutung mineralischer und stickstoffhaltiger Nährsubstanzen für die Hefen und deren Gärfähigkeit.—Brennerei-Zeitg. 1897. XIV. No. 318.—C. f. B. 2. Abt. 1898. IV. 154.—K. J. VIII. 84.
II. Die Haltbarkeit der Hefe.—Brennerei-Zeitg. 1898. XV. 1998.—C. f. B. 2. Abt. 1899. V. 39.
III. Die quantitative Bestimmung der Hefe bei Gärversuchen.—Z. f. Spiritusind. 1897. XX. 106.—Ch. C. 1897. II. 767.—K. J. VIII. 94.
IV. Abkürzung der Gärzeit.—Z. f. Spiritusind. 1897. XX. 97.—K. J. VIII. 129.

Kutscher, Fr.,
I. Die Vibrionen- und Spirillen Flora der Düngerjauche.—Z. f. Hygiene. 1895. XX. 46.—C. f. B. 2. Abt. 1895. I. 645.
II. Z. f. phys. Chem. 1901. XXXII. 59.
III. *Ibid.* 1901. XXXII. 59, 419. 1902. XXXIV. 517, 520.

Kutscher, Fr., u. Lohmann,
I. Z. f. physiolog. Chemie. 1903. XXXIX. 159, 313.

Kuyper,
I. Ann. Mycologici. 1905. III. 52.

Laborde, J.,
I. Sur la casse des vins.—C. R. 1896. CXXIII. 1074.—Ch. C. 1897. I. 447.—K. J. VII. 145.
II. Sur l'absorption d'oxygène dans la casse du vin.—C. R. 1897. CXXV. 248.—Ch.C. 1897. II. 649.—K. J. VIII. 274.
III. De la glycérine dans les vins provenant de raisins atteints de pourriture noble.—Revue de viticulture. 1897, p. 524.—Ch. C. 1898. I. 643.
IV. Sur l'oxydase du Botrytis cinerea.—C. R. 1898. CXXVI. 536.—Ch. C. 1898. I. 746.—K. J. IX. 302.
V. Contribution à l'étude de l'azote contenu dans le vin. — Ann. Inst. Past. 1898. XII. 517.—Ch. C. 1898. II. 734.—K. J. IX. 158.
VI. Ann. Inst. Past. 1897. XI. 1.—Comptes rendus de la Soc. Biologie. 1895. 472.
VII. C. R. 1899. CXXIX. 344.
VIII. Ann. Inst. Past. 1897 [? 1887]. I. 1.

Labourasse, G.,
See Petit et Labourasse.

Lachmann, Johannes,
I. Über Knollen an den Wurzeln der Leguminosen.—Zeitschrift d. Kgl. Lehranstalt und Versuchs-Station Poppelsdorf. 1858. Heft I.—Reproduced later in Biedermann's Centralblatt. 1891. XX. 837.—C. f. B. 1891. X. 90.

Laer, Henri van,
I. Contributions à l'histoire des ferments des hydrates de carbone (Bacille des bières tournées).—Mém. cour. de l'Acad. Royale de Belgique. 1892. XLVII.—C. f. B. 1893. XIII. 129.—Z. g. Br. 1892. XV. 340.—K. J. III. 97.
II. Note sur les fermentations visqueuses. — Extrait des Mémoires publiés par l'Académie Royale de Belgique. 1889. XXXXIII. 36.—German in Z. g. Br. 1890. XIII. 11.—C. f. B. 1890. VII. 308.
III. Studies on Secondary Fermentations and Frets.—Transactions of the Federated Institutes of Brewing. 1894. VII.—K. J. V. 179.
IV. A Few Words on the Occurrence of Furfurol in Alcoholic Liquids.—Journal of the Federated Institutes of Brewing. 1898. IV. 2.—K. J. IX. 161.
V. La question des rapports de l'oxygène avec la levure.—Bulletin de l'association belge des chimistes. 1893. VII. No. 3.—W. f. Br. 1894. XI. 353.—Ch. C. 1894. I. 1083.—K. J. IV. 137.
VI. Recherches sur la composition d'une levure mixte de fermentation haute.—Bulletin de l'Association belge des Chimistes. 1895. IX. 216.—C. f. B. 2. Abt. 1896. II. 91.—K. J. VI. 158.
VII. C. f. B. 2. Abt. 1905. XIV. 550.
VIII. Journ. Federated Inst. Brewing. 1901. VII. 337.
IX. German Patent No. 117303, Dec. 29; 1898.—Abst. in Chem. Centralbl. 1901. I. 352.
X. Bull. de l'Association belge des Chimistes. 1890. XVI. 177.
XI. W. f. Br. 1894. XI. 353.
XII. Moniteur Scient. 1895. 499.

Laer, H. van, u. Denamur,
I. Moniteur Scient. July 1, 1895.

LAFAR, F.,
I. Z. g. Br. 1894. XVII. 10. 1897. XX. 679.
II. Landw. Jahrbücher. 1895. XXIV. 445.
III. C. f. B. 1893. XIII. 684.

LAGERHEIM, G. von,
I. Svensk Farmac. Tidskrift. 1903. No. 18.

LAMANNA, Paolo Antonio,
I. Über die Giftigkeit der Alkohole.—Bollettino chimico farmaceutico. 1899. XXXVIII. 473.—Ch. C. 1899. II. 446.

LANDOLT, H.,
I. Über die Ursache der Nitrifikation der Ammoniaksalze im Erdboden.—Deutsche landw. Presse. 1888. XV. 185.

LANDWEHR, Herm. Ad.,
I. Über Mucin, Metalbumin und Paralbumin.—Z. f. physiolog. Chemie. 1884. VIII. 114.

LANG, M., u. FREUDENREICH, Ed. von,
I. Über Oïdium lactis.—Landw. Jahrbuch d. Schweiz. 1893. VII. 229.—C. f. B. 1894. XVI. 119.—K. J. IV. 184.
See also HESS, SCHAFFER u. LANG.

LANGE, H.,
I. Über den Einfluss verschiedenartiger Stickstoffernährung auf die Hefe.—W. f. Br. 1899. XVI. 49.—C. f. B. 2. Abt. 1899. V. 226.—Ch. C. 1899. I. 699.—K. J. X. 108.
II. Beitrag zur alkoholischen Gärung ohne Hefezellen.—W. f. Br. 1898. XV. 877.—C. f. B. 2. Abt. 1898. IV. 861.—Ch. C. 1898. II. 548.—K. J. IX. 317.
III. Jahrb. d. Versuchs. u. Lehranstalt f. Brauerei in Berlin. 1904. VII. 43.
IV. Chem. Ztg. 1902. XXVI. 200.

LAQUERRIÈRE,
See APOSTOLI et LAQUERRIÈRE.

LAROQUE,
I. C. R. 1852. XXXV. 221.

LASCHÉ, A.,
I. Saccharomyces Joergensenii. — Der Braumeister. Chicago. 1892.—Z. g. Br. 1892. XV. 113.—C. f. B. 1. Abt. 1892. XII. 558.—K. J. III. 144.
II. Zwei rote Mycoderma-Arten. — Der Braumeister. Chicago. 1892. 278.—C. f. B. 1. Abt. 1893. XIII. 485.—K. J. III. 144.
III. Die Mycoderma und die Praxis.—Der Braumeister. 1891. No. 10.—C. f. B. 1. Abt. 1891. X. 192.—K. J. II. 152.
IV. Die amerikanische Braureihefe.—Der Braumeister. Chicago. 1891. 180.—K. J. III. 159.
V. Einfluss verschiedener Wärmegrade auf gewisse Hefearten, sowie deren Verhalten in säurehaltigen und alkalischen Nährsubstanzen. — American Brewer's Review. 1893. VI. 237.—K. J. IV. 174.
VI. Der Braumeister. Feb. 20, 1892.—K. J. III. 144.

LASER, Hugo,
I. Die makroskopische Wasseruntersuchung durch Anwendung von Wasserstoffsuperoxyd.—C. f. B. 1894. XVI. 180.—Ch. C. 1894. II. 854.
II. Z. f. Hygiene. 1891. X. 513.

LAURENT, Emile,
I. Etudes sur la variabilité du bacille rouge de Kiel.—Ann. Inst. Past. 1890. IV. 465.—C.f.B. 1891. IX. 105.—K. J. I. 38.
II. La bactérie de la fermentation panaire. — Bulletins de l'Acad. Royale des Sciences de Belgique. 1885. 3ème série. X. 765.—C. f. B. 1887. I. 504.—Ch. C. 1887. 118.
III. Über den angeblichen Bakterienursprung der Diastase.—Bullet. de l'Acad. Belg. 1886. X. 38.—Ch. C. 1886. 460.
IV. Sur le microbe des nodosités des Legumineuses.—C. R. 1890. CXI. 754.—C. f. B. 1. Abt. 1891. IX. 703.—K. J. I. 129.
V. Note sur les formes-levures chromogènes.—Bulletin de la Soc. Royale de Botanique de Belgique. 1890. XXIX. II. 76.—K. J. I. 39.
VI. Etudes biologiques. Première partie. Recherches physiologiques sur les levures.—Annales de la Soc. belge de Microscopie. 1890. XIV. 29.—K. J. I. 54.
VII. Recherches sur le polymorphisme du Cladosporium herbarum.—Ann. Inst. Past. 1888. II. 603.
VIII. Nutrition hydrocarbonée et formation de glycogène chez la levure de bière.—Ann. Inst. Past. 1888. II. 113.—C. f. B. 1. Abt. 1889. V. 794.
IX. Recherches sur la valeur comparée des nitrates et des sels ammoniacaux comme aliment de la levure de bière et de quelques autres plantes.—Ann. Inst. Past. 1889. III. 362.—C. f. B. 1. Abt. 1889. VI. 411.
X. Ann. Soc. belge de Microscopie. 1890. XXIV. 54.
See also SCHLÖSING et LAURENT.

LAVOISIER, Antoine Laurent,
I. Traité de Chimie. 2ème édition. 1793. I. 159.
II. Œuvres. III. 780.—System der antiphlogistischen Chemie. 1792. 161.

LAWES, J. B., and GILBERT, J. H.,
I. The Sources of the Nitrogen of our Leguminous Crops. — Journal of the Royal Agricultural Society of England. Third Series. Vol. II. Part IV. 1892, p. 657.—C. f. B. 1892. XII. 298.—K. J. III. 191.

LAWROW, D.,
I. Z. f. physiolog. Chemie. 1901. XXXIII. 312.

LAXA, Ottokar,
I. Über einen thermophilen Bacillus aus Zuckerfabriksprodukten.—C.f. B. 2. Abt. 1898. IV. 362.—Ch. C. 1898. II. 53.
II. A. f. Hygiene. 1902. XLI. 119.

Lea, A. S., and Dickinson, W. S.,
I. Action of Rennin and Fibrin Ferment. —Journal of Physiology. 1890. XI. 307.—K. J. I. 175.

Lebbin,
I. Über die Verwendbarkeit von Wasserstoffsuperoxyd in der Nahrungsmittel-Analyse.—Pharm. Ztg. 1897. XXXXII. 148.—Ch. C. 1897. I. 670.
II. Über Ovos, ein neues, aus Hefe hergestelltes Fleischextrakt-Ersatzmittel.—Die mediz. Woche. 1901. 195.—Ch. C. 1901. I. 1381.

Lebrasseur, A.,
See Gathelinau u. Lebrasseur.

Lechartier, G., et Bellamy, F.,
I. Etude sur les gaz produits par les fruits.—C. R. 1869. LXIX. 356, 466. 1872. LXXV. 1203. 1874. LXXIX. 949, 1006. 1875. LXXXI. 1127.

Lecomte, Henri,
I. Les tubercules radicaux de l'Arachis hypogæa L.—C. R. 1894. CXIX. 302. —C. f. B. 2. Abt. 1895. I. 520.

Ledderhose, Georg,
I. Über salzsaures Glycosamin.—Ber. d. D. Chem. Ges. 1876. IX. 1200.
II. Über Chitin und seine Spaltungsprodukte.—Z. f. physiolog. Chemie. 1878. II. 213.

Lederer, L.,
I. Über Buttersäure und den Bacillus subtilis. — Chemiker-Zeitung. 1892. XVI. 252.—C. f. B. 1892. III. 242.

Legros,
See Rechter et Legros.

Lehmann, C. G.,
I. Lehrbuch der physiologischen Chemie. Leipzig. 1842.

Lehmann, Jul.,
I. Über die zur Ernährung der Pflanzen geeignetste Form des Stickstoffes.—Biedermann's Centralblatt für Agriculturchemie. 1875. VII. 403.

Lehmann, Karl Bernhard,
I. Vorläufige Mitteilung über die Desinfektion von Kleidern, Lederwaren, Bürsten und Büchern mit Formaldehyd. —Münchner medizin. Wochenschrift. 1893. No. 30 u. f.—Ch. C. 1894. I. 292.—K. J. IV. 113.
II. Über die Sauerteiggärungen und die Beziehungen des Bacillus levans zum Bacillus coli communis.—C. f. B. 1894. XV. 350.—Ch. C. 1894. II. 102.
III. Qualitative und quantitative Untersuchungen über den Säuregehalt des Brotes.—A. f. Hygiene. 1893. XIX. 363.—C. f. B. 1894. XV. 556.—Ch. C. 1894. I. 518.
IV. Hygienische Studien über Kupfer. VI. — A. f. Hygiene. 1898. XXXI. 279.—Ch. C. 1898. I. 580.

Lehmann, K. B., u. Neumann, R.,
I. Atlas und Grundriss der Bakteriologie. Zwei Bände. With 63 coloured plates. München. 1896. J. F. Lehmann. 15*s.*, bound.—K. J. VII. 1.
Engl. Ed. 1897. London. Baillière, Tindall & Cox. 12*s.* 6*d.*

Lehmann, Victor,
I. Über das Verhalten des Guanins, Xanthins und Hypoxanthins bei der Selbstgärung der Hefe.—Z. f. physiolog. Chemie. 1885. IX. 563.

Leichmann, G.,
I. Über die freiwillige Säuerung der Milch.—Milchzeitung. 1894. XXIII. 523. 1896. XXV. 67.—C. f. B. I. Abt. 1894. XVI. 826.—K. J. V. 239.
II. Über die im Brennereiprozess bei der Bereitung der Kunsthefe auftretende spontane Milchsäuregärung.—C. f. B. 2. Abt. 1896. II. 281.—K. J. VII. 139.
III. Über eine schleimige Gärung der Milch.—Vers.-Stat. 1894. XLIII. 375. —C. f. B. 1894. XVI. 122.—Ch. C. 1894. I. 827.—K. J. V. 216.

Lemaire, J.,
I. Rôle des infusoires et des matières albuminoides dans la fermentation, la germination et la fécondation.—C. R. 1860. LI. 536.
II. De l'acide phénique, de son action sur les végétaux, les animaux, les ferments, les venins, les virus, les miasmes et de ses applications à l'industrie, à l'hygiène, aux sciences anatomiques et à la thérapeutique. 2nd edition. Paris. 1865.

Lendner, A.,
I. The Combined Influence of Light and Medium on the Development of Fungi. —U. S. Department of Agriculture. Experiment Station Record. 1896. VIII.—K. J. VII. 43.
II. Bull. de l'Herbier Boissier. 1903. 3rd Series. III. 362.

Lenz, Wilhelm,
I. Über die Beurteilung von Cognac auf Grund der chemischen Analyse.—Zeitschrift f. öffentl. Chemie. 1899. V. 258. —Ch. C. 1899. II. 539.

Lepeschkin, W. W.,
I. C. f. B. 2. Abt. 1903. X. 105.

Lermer, J. C.,
I. Notiz über das Alkaloid des Bieres. —Dingler's polyt. Journal. 1867. CLXXXIV. 159.

Lesage, Pierre,
I. Recherches expérimentales sur la germination des spores du Penicillium glaucum.—Annales des sciences naturelles. Botanique. 8e série. 1895. I. 309.—K. J. VI. 91.
II. Action de l'alcool sur la germination des spores de champignons. — *Ibid.* 1897. III. 151.—K. J. VII. 45.

III. Recherches physiologiques sur les champignons.—C. R. 1894. CXVIII. 607.—Bot. Ztg. 2. Abt. 1895. LIII. 159.—K. J. V. 78.
IV. C. R. 1902.—Bot. Centralbl. 1902. LXXXIX. 87. 1903. XCII. 94.

LEUBE, W.,
I. Über die ammoniakalische Harngärung. —Virchow's Archiv f. patholog. Anatomie. 1885. C. 540.
II. Über Harnstoff-Ferment. — Deutsch. mediz. Zeitg. VII. 379.—Ch. C. 1886. 459.

LEUCHS,
I. Weinkunde. 1847.

LEUDET, R.,
See WURTZ et LEUDET.

LEUFVÉN, Gust. J.,
I. Einfluss der Melkung auf den Bakteriengehalt der Milch. — Redogörelse för verksamheten vid Ultuna landtbruksinstitut år 1894. 38.—Upsala. 1895. —C. f. B. 2. Abt. 1895. I. 824.

LEVY, A.,
I. Über spontane Milchgerinnung und die biologische Bedeutung des Gerinnungsprozesses.—C. f. B. 1888. III. 421.
II. Die wahre Natur des Kefirs.—Deutsche Med. Ztg. 1886. 783.

LEWANDOWSKI, A.,
I. Über Indol- und Phenolbildung durch Bakterien.—Deutsche mediz. Wochenschrift. 1890. 1186.—K. J. I. 36.

LEWITH,
I. Über die Ursache der Widerstandsfähigkeit der Sporen gegen hohe Temperaturen.—Archiv f. experim. Pathologie u. Pharmakologie. 1889. XXVI. 341.

LEWKOWITSCH, J.,
I. Darstellung rechtsdrehender Mandelsäure aus inaktiver Mandelsäure.—Ber. d. D. Chem. Ges. 1882. XV. 1505.
II. Spaltung der inaktiven Mandelsäure in ihre beiden optisch aktiven Isomeren. —Ber. d. D. Chem. Ges. 1883. XVI. 1568.

LIBORIUS, Paul,
I. Beiträge zur Kenntnis des Sauerstoffbedürfnisses der Bakterien.—Z. f. Hygiene. 1886. I. 115.—Ch. C. 1886. 579.—Bot. Centralbl. 1886. XXVII. 198.

LIEBERMANN, Leo,
I. Über das Nuclein der Hefe und künstliche Darstellung eines Nucleines aus Eiweiss und Metaphosphorsäure.—Ber. d. D. Chem. Ges. 1888. XXI. 598. —Ch. C. 1888. p. 447.
II. Über Nucleine. — Centralblatt f. d. medizin. Wissenschaften. 1889. 210, 497.—Ch. C. 1889. I. 540, 590; II. 335.
III. Nachweis der Metaphosphorsäure im Nuclein der Hefe.—Pflüger's Archiv. 1890. XXXXVII. 155.—Ch. C. 1890. I. 1028.—K. J. I. 32.
IV Ber. d. D. Chem. Ges. 1882. XV. 437, 2553.

LIEBERMANN, L., u. BITTÓ, B. v.,
I. Ein Beitrag zur Chemie der Hefezellen. —Centralblatt f. Physiologie. 1894. VII. 857.—Ch. C. 1894. I. 824.—K. J. V. 115.
II. Nucleinsäure.—Centralblatt f. d. mediz. Wissenschaften. 1893. 465.—Ch. C. 1893. II. 649.

LIEBIG, Johannes,
I. Über die Ursachen des raschen Gerinnens der Milch und die Mittel dasselbe zu verhindern. Dissertation. Heidelberg. 1890.—K. J. I. 84.

LIEBIG, Justus von,
I. Das enträtselte Geheimnis der geistigen Gärung. — Annalen d. Chemie und Pharmacie. 1839. XXIX. 100.
II. Über die Gärung und die Quelle der Muskelkraft. — Sitzungsber. d. bayr. Akademie. 1868 u. 1869.—Annalen d. Chemie und Pharmacie. 1870. CLIII. 1, 137.
III. Liebig's Ann. 1870. CLIII. 8.

LIEBSCHER,
I. Beitrag zur Stickstoff-Frage.—Journal f. Landwirtschaft. 1893. XXXXI. 139. —Ch. C. 1893. II. 94.—K. J. IV. 228.

LIESENBERG, C., und ZOPF, W.,
I. Über den sogenannten Froschlaichpilz (Leuconostoc) der europäischen Rübenzucker- und der javanischen Rohrzuckerfabriken. — Beiträge zur Physiologie und Morphologie niederer Organismen. Edited by W. Zopf. Heft I. Leipzig. 1892. 1. Continuation in Heft 2, 1. —C. f. B. 1892. XII. 659. 1893. XIII. 339.—K. J. III. 89.

LILIENFELD, Leo,
I. Über die Wahlverwandtschaft der Zellelemente zu gewissen Farbstoffen. —Archiv f. Anatomie und Physiologie. 1893. Physiol. Abt. p. 391.

LILIENFELD, Leon, und MONTI, A.,
I. Über die mikrochemische Lokalisation des Phosphors in den Geweben.—Z. f. physiolog. Chemie. 1893. XVII. 410. —K. J. IV. 74.—Rendiconti della Accademia dei Lincei. Roma. 1893. II. 310.—Ch. C. 1893. I. 50.—Bot. Ztg. 1893. 2. Abt. LI. 245.

LIND, K.,
I. Über das Eindringen von Pilzen in Kalkgesteine und Knochen.—Pringsheim's Jahrbücher. 1898. XXXII. 603. Gives the antecedent bibliography. —C. f. B. 2. Abt. 1899. V. 192.

LINDAU, G.,
I. Hedwigia. 1904. XLIII. 306.
II. Hyphomycetes in Rabenhorst's Kryptogamenflora Deutschlands. 2nd Ed. 1904. I. Abt. 8.
III. In Engler-Prantl, Natürl. Pflanzenfamilien. 1900. Teil 1. Abt. 1. 383, 416.

LINDET, L.,
I. Sur l'oxydation du tannin de la pomme à cidre.—Le Cidre. 1893, p. 150.—C. R. 1895. CXX. 370.—Ch. C. 1895. I. 656.
II. Sur le dosage des bases dans les flegmes industriels.—C. R. 1888. CVI. 280.
III. Bull. Soc. Chim. 1889. 3e Série. II. 195.
IV. C. R. 1891. CXII. 102, 663.
V. *Ibid.* 1888. CVII. 182.
VI. *Ibid.* 1890. CXI. 236.

LINDET et MARSAIS,
I. Ann. de la Brasserie. 1905. 3.

LINDNER, G.,
I. Studien über die Biologie u. hygienische Bedeutung der im Essig lebenden Nematoden.—C. f. B. 1889. VI. 633.

LINDNER, Paul,
I. Über ein neues, in Malzmaischen vorkommendes, Milchsäure bildendes Ferment.—W. f. Br. 1887. IV. 437.—C. f. B. 1. Abt. 1887. II. 340.—Ch. C. 1887. 1507.
II. Die Sarcina-Organismen der Gärungsgewerbe. Dissert. Berlin. 1888.—C. f. B. 1. Abt. 1888. IV. 427.
III. Die Ursache des langen Weissbieres. —W. f. Br. 1889. VI. 181.
IV. Die Weinsäurekur für sarcinahältige Zeuge.—W. f. Br. 1895. XII. 316.—C. f. B. 2. Abt. 1896. II. 36.
V. Das Langwerden der Würze durch Dematium pullulans.—W. f. Br. 1888. V. 290.—Z. g. Br. 1888. XI. 225.—C. f. B. 1. Abt. 1888. III. 750.
VI. Mikroskopische Betriebskontrolle in den Gärungsgewerben. 2nd Ed. Berlin. 1898. Parey.—3rd Ed. 1901. 17*s*.—C. f. B. 2. Abt. 1902. IX. 936.
VII. Fruchtäther-Bildung durch Hefen in Grünmalz und in Würzen.—W. f. Br. 1896. XIII. 552.—Z. g. Br. 1896. XIX. 675.—Ch. C. 1896. II. 273.—K. J. VII. 100.
VIII. Beobachtungen über die Sporen- und Glycogenbildung einiger Hefen auf Würzegelatine. Die Blaufärbung der Sporen von Schizosaccharomyce octosporus durch Jodlösung.—C. f. B. 2. Abt. 1896. II. 537.—Ch. C. 1896. II. 938.—K. J. VII. 99.
IX. Über Durchwachsungen an Pilzmycelien.—Ber. d. D. Bot. Ges. 1887. V. 153. Gives the antecedent bibliography.
X. Hefezellen als Amöbennahrung und amöbenförmige Hefezellen.—W. f. Br. 1889. VI. 1069.—Z. g. Br. 1890. XIII. 45.—Ch. C. 1890. I. 45.
XI. Das Vorkommen von Amöben im Gärungsbetriebe.—W. f. Br. 1896. XIII. 1231.—K. J. VII. 117.
XII. Monilia variabilis, eine formenreiche und rassenspaltige neue Pilzart.—W. f. Br. 1898. XV. 209.—C. f. B. 2. Abt. 1898. IV. 931.
XIII. Die Vegetationsverhältnisse im untergärigen Bier während der Vergärung.—W. f. Br. 1895. XII. 477.—C. f. B. 2. Abt. 1895. I. 890.—K. J. VI. 163.
XIV. Saccharomyces farinosus und Saccharomyces Bailii. Zwei neue Hefenarten aus Danziger Jopenbier.—W. f. Br. 1894. XI. 153.—Ch. C. 1894. I. 609.—K. J. V. 37.
XV. Die Tröpfchenkultur und die Bedeutung des Mikroskopes in der Brauerei. —W. f. Br. 1894. XI. 697. 1896. XIII. 80.—K. J. V. 10.
XVI. Empfiehlt es sich an Stelle von Kräusen in gewissen Fällen das Bier im Lagerfass nur mit Hefe zu behandeln? —W. f. Br. 1898. XV. 692.—K. J. IX. 123.
XVII. Bemerkungen zum Moeller'schen Filter.—W. f. Br. 1893. X. 775.—K. J. IV. 33; III. 157.
XVIII. Ergebnisse einiger Luftuntersuchungen von Brauereien nebst Bemerkungen zu Hansen's Methode der Luftanalyse.—W. f. Br. 1888. V. 877.—Z. g. Br. 1888. XI. 490.
XIX. Welches sind die besten Hefenrassen zur Vergärung von Dickmaischen und welche eignen sich hervorragend zur Erzielung hoher Hefenausbeuten in der Presshefefabrikation. Mitteilung von Züchtungsresultaten von 37 Reinhefen. —Z. f. Spiritusind. 1890. XIII. Ergänzungsheft. 29.—K. J. I. 60.
XX. Die Resultate von 99 Gärversuchen in tabellarischer Darstellung mit dazugehörigen Erläuterungen.—W. f. Br. 1894. XI. 381.—K. J. V. 109.
XXI. Die Einzellkultur im hängenden Tropfen. Gealterte Zellen in frischer Würze. Abnormale Zellformen und Querwandbildungen bei Betriebshefen. —W. f. Br. 1893. X. 1354.—K. J. IV. 13.
XXII. Über die Erkennung der Heferassen und ihre photographische Darstellung.—W. f. Br. 1891. VIII. 815.—C. f. B. 1892. XII. 250.—K. J. II. 149.
XXIII. Das Wachstum der Hefen auf festen Nährböden.—W. f. Br. 1893. X. 690.—C. f. B. 1893. XIV. 372. —K. J. IV. 44.
XXIV. Atlas der mikroskopischen Grundlagen der Gärungskunde. 111 Tafeln. Berlin. 1903. Parey.
XXV. Wie konserviert man obergärige Hefe am besten?—Jahrbuch d. Versuchs- u. Lehranstalt f. Brauerei in Berlin. 1899. II. 235.—K. J. X. 173.
XXVI. Der Hefereinzuchtapparat unseres Vereinslaboratoriums.—W. f. Br. 1888. V. 917. 1892. IX. No. 20. Beilage.
XXVII. Hat sich Reinzuchthefe für Weissbierbrauereien bewährt. In welcher Weise wäre die Einführung zu organisieren?—W. f. Br. 1898. XV. 717.—K. J. IX. 102.

XXVIII. W. f. Br. 1901. XVIII. 130.
XXIX. Mikroskopische Betriebskontrolle, etc. 3rd Ed. 1901. 327.
XXX. W. f. Br. 1893. X. 1298.
XXXI. Mikroskopische Betriebskontrolle. 1895, and 3rd Ed., 1901.
XXXII. Jahrb. d. Versuchs- und Lehranstalt, etc., in Berlin. 1904. VII. 448.
XXXIII. Mikroskopische Betriebskontrolle, etc. 3rd Ed. 1901. 314, 317–319.—Atlas der Mikroskopischen Grundlagen der Gärungskunde. Berlin. Plates 33 and 35.
XXXIV. W. f. Br. 1887. IV. 853.
XXXV. *Ibid.* 1900. XVII. 713 *et seq.*
XXXVI. Mikroskopische Betriebskontrolle. 4th Ed. Berlin. 1905. 421.
XXXVI*a*. *Ibid.*, p. 424.
XXXVI*b*. *Ibid.*, p. 427.
XXXVII. *Ibid.*, p. 422.
XXXVIII. W. f. Br. 1903. XX. 505.
XXXIX. C. f. B. 2. Abt. 1895. I. 782.
XL. Mikroskopische Betriebskontrolle. 4th Ed. Berlin. 1905.
XLI. W. f. Br. 1894. XI. 1312.
XLII. Mikroskopische Betriebskontrolle. 3rd Ed. 1901. 109, 380.
XLIII. W. f. Br. 1897. XIV. 546.
XLIV. Mikroskopische Betriebskontrolle. 3rd Ed. 1901. 385.
XLV. W. f. Br. 1900. XVII. 713.
XLVI. *Ibid.* 1893. X. 1298.
XLVII. Mikroskopische Betriebskontrolle, etc. 3rd Ed. 1901. 358.

See also Fischer und Lindner.

Linossier, G.,
I. Action de l'acide sulfureux sur quelques champignons inférieurs et en particulier sur les levures alcooliques.—Ann. Inst. Past. 1891. V. 170.—K. J. II. 93.
II. C. R. 1891. CXII. 489, 807.

Lintner, Karl, der ältere,
I. Zymotechnische Rückblicke auf das Jahr 1877.—Z. g. Br. 1878. I. 17.
II. Desgl. auf das Jahr 1878.—Z. g. Br. 1879. II. 2.
III. Altes und Neues über Bierbrauerei.—Z. g. Br. 1880. III. 2 *et seq.* 1881. IV. 11 *et seq.*
IV. Zymotechnische Rückblicke auf das Jahr 1882.—Z. g. Br. 1883. VI. 397.

Lintner, C. J.,
I. Handbuch der landwirtschaftlichen Gewerbe.—Berlin. 1893. Parey.
II. Über die Entstehung von Dextrose aus der Stärke durch fermentative Prozesse.—Z. g. Br. 1892. XV. 123.—Ch. C. 1892. I. 740.—K. J. III. 255.
III. Studien über die Selbstgärung der Hefe.—C. f. B. 2. Abt. 1899. V. 793.—Ch. C. 1900. I. 54.—K. J. X. 106.
IV. Zur Kenntnis der sogen. stickstofffreien Extraktstoffe in der Gerste, bez. im Malze und Biere. — Zeitschrift f. angewandte Chemie. 1890. 519.—Z. g. Br. 1890. XIII. 472. 1891. XIV. 81.—Ch. C. 1890. II. 804.
V. Zur Kenntnis der Stickstoffaufnahme durch die Hefe bei der Gärung.—W. f. Br. 1884. I. 3.—Z. g. Br. 1884. VII. 143.
VI. Z. g. Br. 1892. XV. 213.
VII. Chem. Ztg. 1899. XXIII. 851.
VIII. Z. f. angew. Chem. 1896. 538.
IX. Forschungsberichte über Lebensmittel, etc. 1894. I. 275.
X. Z. g. Br. 1892. XV. 106. 1894. XVII. 414.
XI. *Ibid.* 1892. XV. 6.
XII. Z. f. angew. Chem. 1892. 328.

Lintner, C. J., und Düll, G.,
I. Über die chemische Natur des Gerstengummi. — Zeitschrift f. angew. Chemie. 1891. 538.—Ch. C. 1891. II. 799.
II. Ber. d. D. Chem. Ges. 1893. XXVI. 2533.

Lintner, C. J., und Kröber, E.,
I. Ber. d. D. Chem. Ges. 1895. XXVIII. 1050.

Lippmann, Edmund O. von,
I. Die Chemie der Zuckerarten. 2nd Ed. 1895. Braunschweig. Fr. Vieweg u. Sohn.
II. *Ibid.* 1904.
III. Ber. d. D. Chem. Ges. 1887. XX. 1001.
IV. *Ibid.* 1903. XXXVI. 331.
V. Die Chemie der Zuckerarten. 1904. 1623.

Lister, Joseph,
I. A Further Contribution to the Natural History of Bacteria and the Germ Theory of Fermentative Changes. — Quarterly Journal of Microscopical Science. 1873. XIII. 380.
II. On the Lactic Fermentation and its Bearing on Pathology.—Transactions of the Pathological Society of London. 1878. XXIX.

Lloyd, E. J.,
I. Observations on Cheddar Cheesemaking.—Reports for 1891, 1892 and 1893.

Loder,
I. A. f. Hygiene. 1902. XLII. 107.
II. *Ibid.*, p. 152.

Loé, W.,
I. Enthält das Malz ein peptonisierendes Enzym ?—Z. g. Br. 1899. XXII. 212.—Ch. C. 1899. I. 1248.

Loeffler, Friedrich,
I. Eine neue Methode zum Färben der Mikroorganismen im besonderen ihrer Wimperhaare und Geisseln.—C. f. B. 1889. VI. 209.
II. Weitere Untersuchungen über die Beizung und Färbung der Geisseln bei den Bakterien.—C. f. B. 1890. VII. 625.—K. J. I. 9.
III. Über Bakterien in der Milch.—Berliner klin. Wochenschrift. 1887. 607.—C. f. B. 1887. II. 524.

Loew, E.,
I. Über Dematium pullulans.—Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1867. VI. 464.
II. Zur Entwicklungsgeschichte von Penicillium.—*Ibid.* 1870. VII. 472.
III. Zur Physiologie niederer Pilze.—Verh. der K. K. zoolog.-botan. Ges. in Wien. 1867. XVII. 643.
IV. Jahr. wiss. Bot. 1869–70. VII. 472.
V. *Ibid.* 1867. VI. 467.

Loew, Oskar,
I. Physiologische Notizen über Formaldehyd.—Ch. C. 1889. I. 90.
II. Ein natürliches System der Giftwirkung. München. 1893.—C. f. B. 1893. XIV. 234.
III. Katalytische Bildung von Ammoniak aus Nitraten.—Ber. d. D. Chem. Ges. 1890. XXIII. 675.—K. J. I. 35.
IV. Miso und Natto.—Mitteilungen der D. Ges. f. Natur- und Völkerkunde Ostasiens in Tokio. Heft 57. — Ch. C. 1896. II. 186.
V. Über die Ernährungsweise des nitrifizierenden Spaltpilzes Nitromonas.—Botan. Centralblatt. 1891. No. 20.—C. f. B. 1891. IX. 691.—Ch. C. 1891. II. 549. K. J. II. 209.
VI. Bemerkung zu einer Abhandlung von K. Yoshimura.—Ch. C. 1896. I. 57.
VII. Zur Frage der Vertretbarkeit von Kaliumsalzen durch Rubidiumsalze bei niederen Pilzen. — Bot. Centralblatt. 1898. LXXIV. 202.—Ch. C. 1898. II. 41.—K. J. IX. 55.
VIII. Über den Nachweis des Lecithins.—Pflüger's Archiv f. d. gesamte Physiologie. XIX. 342.
IX. Münchn. medic. Wochenschr. 1892. XXXIX. 370.
X. Chem. Ztg. 1900. XXIV. 1137.

Lohmann, W.,
I. Über den Einfluss des intensiven Lichtes auf die Zellteilung bei Saccharomyces cerevisiæ und anderen Hefen. Dissert. Rostock. 1896.—C. f. B. 2. Abt. 1897. III. 369.—K. J. VII. 90.

Loiseau, D.,
I. Sur une nouvelle substance organique cristallisée.—C. R. 1876. LXXXII. 1058.
II. Sur la fermentation de la raffinose, en présence des diverses espèces de levure de bière.—C. R. 1889. CIX. 614.—Z. g. Br. 1889. XII. 483.—Ch. C. 1889. II. 869.

Longard, K.,
See Buchner, Longard und Riedlin.

Lookeren-Campagne, C. J. van,
I. Bericht über Indigo-Untersuchungen, ausgeführt an der Versuchs-Station zu Klatten auf Java.—D. landw. Vers.-Stat. 1894. XXXXIII. 401.—Ch. C. 1894. I. 1000.
II. Über Indigobildung aus Pflanzen der Gattung Indigofera.—D. landw. Vers.-Stat. 1895. XXXXVI. 249.—Ch. C. 1896. I. 113.—K. J. V. 289.

Lopriore, G.,
I. Über einen neuen Pilz, welcher die Weizensaaten verdirbt. — Deutsche landw. Presse. 1891. 321.—Ber. d. D. Bot. Ges. 1892. X. 72.—Zeitschr. f. Pflanzenkrankheiten. 1891. I. 1892. II. 172. 1895. V. 242. — Bot. Ztg. 1895. II. 322.
II. Über die Einwirkung der Kohlensäure auf das Protoplasma der lebenden Pflanzenzelle. — Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1895. XXVIII. 531.—Gives the antecedent bibliography.—Bot. Ztg. 2. Abt. 1896. LIV. 132.

Lubavin, N.,
I. Über die künstliche Pepsin-Verdauung des Caseins und die Einwirkung von Wasser auf Eiweisssubstanz.—Hoppe-Seyler's Mediz.-chem. Untersuchungen. 1871. Heft 4. 463.
II. Über das Nuclein aus dem Casein der Kuhmilch.—Ber. d. D. Chem. Ges. 1877. X. 2237. 1879. XII. 1021.

Lubawin,
See Lubavin.

Lucas, Fr.,
I. Das Obst und seine Verwertung. Stuttgart. Ulmer. 6*s.*, bound.

Lucet,
I. De l'Aspergillus fumigatus chez les animaux. Paris. 1899.

Ludwig, Ernst,
I. Über das Vorkommen des Trimethylamins im Wein.—Sitzgsber. der Kais. Akademie d. Wiss. zu Wien. Mathem.-naturw. Klasse. 1867. LVI. 2. Abt. 287.—Ch. C. 1867. II. 911.

Ludwig, Friedrich,
I. Die bisherigen Untersuchungen über photogene Bakterien.—C. f. B. 1887. II. 372. Gives all the bibliography prior to 1887.—Ch. C. 1887. 1510.
II. Die Genossenschaften der Baumflussorganismen. Sammelreferat.—C. f. B. 2. Abt. 1896. II. 337. Gives the bibliography up to 1896. — K. J. VII. 25.

Lübbert, A.,
I. Über die Natur der Giftwirkung peptonisierender Bakterien der Milch.—Z. f. Hygiene. 1896. XXII. 1.—Ch. C. 1896. II. 47.—K. J. VII. 194.

Lüdersdorff,
I. Pogg. Ann. 1846. LXVII. 408.

Luksch, Ludwig,
I. Zur Differentialdiagnose des Bacillus typhi abdominalis (Eberth) und des Bacterium coli commune (Escherich).—C. f. B. 1892. XII. 427. — K. J. III. 79.

LUMSDEN, J. S.,
See FRANKLAND and LUMSDEN.

LUNDE, H. P.,
See FRIIS, LUNDE u. STORCH.

LUNDSTRÖM, C.,
I. Die Zersetzung von Harnstoff durch Mikroben. Helsingfors. 1890.—K. J. II. 260.

LUTZ, L.,
I. Bull. de la Soc. Mycol. de France. 1899. XV. 68, 157.
II. *Ibid.* 1905. XXI. 131.

MAAS, H.,
I. Über Fäulnisalkaloide. — Fortschritte der Medizin. 1883. I. 373. 1884. II. 729.—Ch. C. 1883. p. 712. 1884. 975.

MAASSEN, Alb.,
I. Arb. Kais. Ges.-Amt. 1901. XXIII. 21.

MAASSEN, Albert,
I. Beiträge zur Ernährungsphysiologie der Spaltpilze. Die organischen Säuren als Nährstoffe und ihre Zersetzbarkeit durch die Bakterien. — Arbeiten a. d. K. Gesundheits-Amte. 1895. XII. 390.—Ch. C. 1896. I. 655.

MACAGNO, J.,
I. Über die Wirkung des in den umgeschlagenen Weinen enthaltenen Fermentes auf gesunde Weine.—Biedermann's Centralbl. 1879. VIII. 310 nach Le Stazioni sperimentali agrarie italiane. 1877. VI. 79.

MACALLUM, A. B.,
I. On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells.—Quarterly Journal of Microscopical Science. 1895. XXXVIII. II. 175.
II. On the Demonstration of Iron in Chromatin by Microchemical Methods. —Proceedings of the Royal Society. London. 1892. L. 277.

MACALPINE,
I. Agricult. Gazette of N.S. Wales. May, 1896.

MACÉ,
I. Traité pratique de Bactériologie. First edition, 1889; second edition, 1892. Paris. Baillière et fils.—C. f. B. 1893. XIII. 484.
II. Archiv de Parasitologie. 1903. VII. 313. Also issued as a thesis, Paris, 1903, "Etudes sur les Mycoses experimentales."

MACFADYEN, A.,
I. Chemisch-bakteriolog. Untersuchungen über einen Euterentzündung und Käseblähung bewirkenden Bacillus (Bacillus Guillebeau c.).—Landw. Jahrbüch d. Schweiz. 1890. IV. 64.—K. J. II. 196.

MACFADYEN, Allan, MORRIS, G. Harris, und ROWLAND, Sidney,
I. Über ausgepresstes Hefezellplasma. I. — Ber. d. D. Chem. Ges. 1900. XXXIII. 2764.—C. f. B. 2. Abt. 1901. VII. 25.—Ch. C. 1900. II. 1028.

MACFADYEN, A., NENCKI, M., und SIEBER, N.,
I. Untersuchungen über die chemischen Vorgänge im menschlichen Dünndarm. —Archiv f. experim. Pathol. u. Pharmakologie. 1891. XXVIII.—Centralbl. f. Physiologie. 1891. V. 199.—C. f. B. 1891. X. 82.—Ch. C. 1891. II. 387.

MACGREGOR, J.,
See FRANKLAND and MACGREGOR.

MACH, Edmund,
I. Botrytis cinerea und der Rauchgeschmack des Weines. — Weinlaube. 1872. IV. 215.

MACH, Edmund, und PORTELE, K.,
I. Über die schwere Vergärbarkeit und die Zusammensetzung des Preisselbeersaftes. —D. landw. Vers.-Stat. 1890. XXXVIII. 69.—Ch. C. 1890. II. 633.—K. J. I. 63.
II. Nachweis und quantitat. Bestimmung von Milchsäure u. Buttersäure in Weinen, die aus verschlämmten Trauben in verschiedener Weise hergestellt wurden.—D. landw. Vers.-Stat. 1890. XXXVII. 305.
III. Über die Gärung von Trauben- und Apfelmost mit verschiedenen rein gezüchteten Hefearten.—D. landw. Vers.-Stat. 1892. XLI. 233.—Ch. C. 1893. I. 218.—K. J. III. 147.

MACHELEIDT,
I. W. f. Br. 1898. XV. 365.

MAERCKER, Max,
I. Handbuch der Spiritusfabrikation. 7. Ed. Berlin. 1898. 22*s.*, bound.
II. *Ibid.* 8th Ed. 1903. 71.
III. *Ibid.* 392.
IV. *Ibid.* 762.
V. *Ibid.* 496.

MAGGIORA, A.,
I. Ricerche quantitative sui microorganismi del suolo con speciale riguardo all' inquinazione del medesimo.—Estrad. dal Giorn. d. R. Accad. di medicina. Torino. 1887. No. 3.—C. f. B. 1887. I. 677.—Ch. C. 1887. 1116.
II. Über die Zusammensetzung des überreifen Käses.—A. f. Hygiene. 1892. XIV. 217.—K. J. III. 188.
See also PERRONCITO e MAGGIORA.

MAJSTOROVIĆ, R.,
See ZEGA und MAJSTOROVIĆ.

MALERBA, P.,
I. Untersuchungen über die Natur der von dem Gliscrobacterium gebildeten schleimigen Substanz.—Z. f. physiolog. Chemie. 1891. XV. 539.—K. J. II. 223.

MALERBA, P., e SANNA-SALARIS, G.,
I. Ricerche sul Gliscrobatterio.—Rendiconto della R. Accademia delle Scienze fis. e mat. di Napoli. 1888. Fasc. 6.—C. f. B. 1888. IV. 486.

Malfatti, Hans,
I. Zur Chemie des Zellkerns.—Berichte d. naturwissenschaftlich-medizinischen Vereines in Innsbruck. XX. Jahrgang. 1891.—92. p. IX.

Malfitano, G.,
I. La protéolyse chez l'Aspergillus niger. —Ann. Inst. Past. 1900. XIV. 60, 420.—C. f. B. 2. Abt. 1900. VI. 472. —Ch. C. 1900. II. 391.

Mallèvre, A.,
See Bertrand et Mallèvre.

Malpighi, Marcello,
I. Opera omnia. Leiden. 1687. II. 126.

Malvezin, F.,
I. Manuel de Pasteurisation des Vins et traitement de leur maladies. Bordeaux. 1899. 6*s.* 6*d.*

Man, C. de,
See Forster u. de Man.

Manassein, Marie von,
I. Beiträge zur Kenntnis der Hefe und zur Lehre von der alkoholischen Gärung.—Wiesner's Mikroskopische Untersuchungen. 1872. Stuttgart. 116.
II. Zur Frage von der alkoholischen Gärung ohne lebende Hefezellen.—Ber. d. D. Chem. Ges. 1897. XXX. 3061.—Ch. C. 1898. I. 395.—K. J. VIII. 284.

Manetti, L., und Musso, G.,
I. Über die Zusammensetzung und die Reife des Parmesankäses.—D. Landw. Versuchsstationen. 1878. XXI. 211.

Mangin, Louis,
I. Sur la constitution de la membrane des végétaux.—C. R. 1888. CVII. 144.—Ch. C. 1888. 1100.—Bot. Ztg. 1889. XXXXVII. 450.
II. Observations sur la constitution de membrane chez les Champignons.—C. R. 1893. CXVII. 816.
III. Sur l'emploi du rouge de ruthénium en Anatomie végétale.—C. R. 1893. CXVI. 653.—Ch. C. 1893. I. 789.
IV. Recherches anatomiques sur la distribution des composés pectiques chez les végétaux.—Journal de Botanique. 1893.
See also Bonnier et Mangin.

Mann, Harold,
I. Action de certaines substances antiseptiques sur la levure.—Ann. Inst. Past. 1894. VIII. 785.—C. f. B. 2. Abt. 1895. I. 521.—Ch. C. 1895. I. 93.—K. J. V. 91.

Marcano, V.,
I. Fermentation de la fécule.—C. R. 1882. LXXXXV. 345, 856.—Ch. C. 1882. 641, 806.
II. Sur la panification.—C. R. 1883. LXXXXVI. 1733.—Ch. C. 1883. 548.
III. Sur la formation de quantités notables d'alcool dans la fermentation panaire.—C. R. 1883. LXXXXVII. 1070.—Ch. C. 1883. 808.
See also Müntz u. Marcano.

Marchal, Emile,
I. Sur la production de l'ammoniaque dans le sol par les microbes.—Bulletin de l'Acad. Royale de Belgique. 1893. XXV. 727. — Naturw. Rundschau. 1893. VIII. 601.—Ch. C. 1894. I. 97.—K. J. IV. 238, 239.
II. The Production of Ammonia in the Soil.—Agricult. Science. 1894. VIII. 574.—C. f. B. 2. Abt. 1895. I. 753.—Biedermann's Centralbl. 1895. XXIV. 519.—Ch. C. 1895. II. 839.
III. Contribution à l'étude microbiologique de la maturation des fromages mous.—Annales de la Société belge de microscopie. 1895. XIX.—C. f. B. 2. Abt. 1895. I. 506.

Marchand, Richard Felix,
I. Über die Zusammensetzung der Milch des Kuhbaumes (Palo de vaca). — Journal für prakt. Chemie. 1840. XXI. 45.

Marckwald,
I. Ber. d. D. Chem. Ges. 1902. XXXV. 1595.

Mariani, Giovanni,
I. Über die Gegenwart und die Menge des Kupfers in dem Parmesan-Käse (formaggio di grana lombardo).—Stazioni sperimentali agrarie italiane. 1889. XVII. — Milchzeitung. 1889. XVIII. 1033.

Marischler, Julius,
I. Klinische Untersuchungen über die Wirkung der an Aldehyd gebundenen schwefligen Säure im Weine.—Wiener klinische Wochenschrift. 1896. IX. 711.—Ch. C. 1896. II. 548.—Vergl. Österr. Chemiker-Zeitg. 1899. II. 33. —Ch. C. 1899. I. 571.

Marpmann, G.,
I. Über die Erreger der Milchsäuregärung.—Ergänzungsheft z. Centralblatt f. allgem. Gesundheitspflege. 1886. II. 117.—Ch. C. 1886. 344.
II. Bakteriologische Mitteilungen.—C. f. B. 1. Abt. 1897. XXII. 122.
III. Über schwarze Pilzwucherungen in offizinellen Flüssigkeiten und über eine neue Hefe-Spezies: Saccharomyces niger.—Archiv der Pharmacie. 1886. CCXXIV. 705.
IV. Käsegärung und Käsepilze.—Pharmac. Centralhalle. 1893. XXXIV. 76.
V. Über die biochemische Arsenreaktion. *Ibid.* 1900. XXXXI. 666.—Ch. C. 1900. II. 1187.
VI. Centralbl. f. allgem. Gesundsheitspflege. 1886. II. 422.
VII. Z. f. angewandte Mikroskopie. 1896. II. 68.
VIII. Pharmac. Ztg. 1903. XLVII. 1010.

MARSCHALL,
I. Über die Zusammensetzung des Schimmelpilzmycels.—A. f. Hygiene. 1897. XXVIII. 16.—C. f. B. 2. Abt. 1897. III. 154.—Ch. C. 1897. I. 115.—K. J. VIII. 42.

MARTIN, C. J.,
I. Journ. of Physiology. 1896. XX. 364.

MARTIN, C. J., and CHAPMAN,
I. Proc. Physiological Soc. June 11, 1898.

MARTINAND, V.,
I. Action de l'air sur le moût de raisin et sur le vin.—C. R. 1895. CXX. 1426; CXXI. 502.—Ch. C. 1895. II. 324, 1100.
II. Influence des rayons solaires sur les levures que l'on rencontre à la surface des raisins.—C. R. 1891. CXIII. 782. —Ch. C. 1892. I. 212.—K. J. II. 127.

MARTINAND, V., et RIETSCH, M.,
I. Des microorganismes que l'on rencontre sur les raisins mûrs et de leur développement pendant la fermentation. —C. R. 1891. CXII. 736.—K. J. II. 128.
II. C. R. 1891. LXII. 763. [*Sic* in the German; LXII. is a misprint for CXII., and 763 should perhaps be 736.]

MARTINY, Benno,
I. Die Milch, ihr Wesen und ihre Verwertung. Zwei Bände. Danzig. 1871. Kafemann. With about 1300 bibliographical references.
II. Das Verarbeiten erhitzter Milch.—Zeitschrift für Fleisch- und Milch-Hygiene. 1893. III. 9.—K. J. IV. 201.

MARX, Louis,
I. Les levures des vins.—Moniteur scientifique. 1888. No. 563.—Z. g. Br. 1888. XI. 522.—C. f. B. 1. Abt. 1889. V. 313.—Ch. C. 1889. I. 47.

MASSART, Jean,
I. Sensibilité et adaption des organismes à la concentration des solutions salines. —Archives de biologie. Liège. 1889. IX. 515.
II. Recherches sur les organismes inférieurs.—Bulletin de l'Académie Royale de Belgique. 1888, 11. 1891. (3.) XXII.—C. f. B. 1. Abt. 1892. XI. 566.—K. J. III. 60.
III. La loi de Weber vérifiée pour l'héliotropisme d'un Champignon.—Bulletin de l'Acad. Roy. de Belgique. 1888. (3.) XVI. No. 12.
IV. La sensibilité tactile chez les organismes inférieurs.—Journal de Médecine de Bruxelles. 1891.—C. f. B. 1. Abt. 1892. XI. 566.

MASSON,
See BALLAND et MASSON.

MASTBAUM, H.,
I. Z. f. Unter. d. Nahrungs- u. Genussmittel. 1903. VI. 49.

MATHEW, W. de Vere,
See SALOMON und MATHEW.

MATHIEU, L.,
I. Rev. de Viticulture. 1898. X. 155.
II. W. f. Br. 1896. XXIII. 334.

MATTERN, J.,
I. Ber. ü. d. 7 Vers. d. freien Verunigung bayr. Vertreter d. angew. Chemie in Speyer. Berlin. 1889.—Abst. in Z. g. Br. 1889. XII. 188.

MATTHEWS, Charles George,
I. On the Red Mould of Barley.—Journal of the Royal Microscop. Society. London. 1883. Ser. 2. Vol. III., p. 321.
II. The Nutrition of Yeast.—Journal of the Federated Institutes of Brewing. 1897. III. 369.—K. J. VIII. 84.
III. The Brewer's Guardian. 1887.—Abst. in W. f. Br. 1887. IV. 380.

MAUREA, G.,
I. Über eine bewegliche Sarcine.—C. f. B. 1892. XI. 228.—K. J. III. 44.

MAXIMOW,
I. Ber. d. D. Bot. Ges. 1904. XXII. 225.

MAYER, Adolf,
I. Untersuchungen über die alkoholische Gärung, den Stoffbedarf und den Stoffwechsel der Hefepflanze. Heidelberg. 1869.—Poggendorff's Annalen. 1871. CXXXXII. 293.—Dingler's polyt. Journal. 1871. CCI. 69.
II. Studien über die Milchsäure-Gärung. —Z. f. Spiritusind. 1891. XIV. 183.—C. f. B. 1892. XII. 99.—K. J. II. 173.
III. Neue Beiträge zur Kenntnis der Wirkung des Labfermentes.—D. Landw. Versuchs-Stationen. 1882. XXVII. 247.
IV. Über die Nägeli'sche Theorie der Gärung ausserhalb der Hefezellen.—Zeitschrift für Biologie. 1882. XVIII. 522.—Z. g. Br. 1882. V. 473.
V. Über den Bedarf des Hefepilzes (Saccharomyces cerevisiæ) an Aschebestandteilen.—D. Landw. Versuchs-Stationen. 1869. XI. 443.
VI. Über den Einfluss der Sauerstoffzufuhr auf die Gärung.—Ber. d. D. Chem. Ges. 1880. XIII. 1163.
VII. Studien über die alkoholische Gärung.—D. Landw. Versuchs-Stationen. 1873. XVI. 277.
VIII. Über den Einfluss des Sauerstoffzutritts auf die alkoholische Gärung.—D. Landw. Versuchs-Stationen. 1880. XXV. 301.—Z. g. Br. 1880. III. 592.
IX. Saccharomyces cerevisiæ und der freie Sauerstoff.—Ber. d. D. Chem. Ges. 1874. VII. 579.
X. Untersuchungen ü. d. alkoholische Gärung. Heidelberg. 1869. 47–51.
XI. Die Lehre v. d. chemischen Fermenten oder Enzymologie. 1882. 64.
XII. *Ibid.* 20.
XIII. *Ibid.* 30.
XIV. *Ibid.* 41.
XV. *Ibid.* 70.
XVI. *Ibid.* 100.

Mazé, P.,
I. Ann. Inst. Past. 1903. XVII. 11.
II. C. R. 1902. CXXXIV. 191; CXXXV. 113.
III. *Ibid.* 1904. CXXXVIII. 1514.

Mazé et Perrier,
I. Ann. Inst. Past. 1904. XVIII. 553.
II. C. R. 1904. CXXXIX. 311.

Medicus u. Immerheiser,
I. Z. f. analyt. Chem. 1891. XXX. 665.

Mège-Mouriès,
I. Recherches chimiques sur le froment, sa farine et sa panification.—C. R. 1856. XXXXII. 1122. 1857. XXXXIV. 40, 449. 1858. XXXXVI. 126.

Mehring,
I. Z. f. physiolog. Chemie. 1881. V. 196.

Meisenheimer,
I. Z. f. physiolog. Chemie. 1903. XXXVII. 518.

Meissl,
I. J. f. prak. Chem. 1880. XXII. 100.

Meissner, E.,
I. Akkomodationsfahigkeit einiger Schimmelpilze. Dissert. Leipzig. 1903.

Meissner, Richard,
I. Über eine neue Spezies von Eurotium Aspergillus.—Bot. Ztg. 2. Abt. 1897. LV. 337.
II. Studien über das Zähewerden von Most und Wein.—Landwirtschaftl. Jahrbücher. 1898. XXVII. 715.—C. f. B. 2. Abt. 1899. V. 232. 1900. VI. 344.—Ch. C. 1899. I. 71. 1900. II. 218.
III. Über das Auftreten und Verschwinden des Glycogens in der Hefenzelle.—C. f. B. 2. Abt. 1900. VI. 517.—Ch. C. 1900. II. 771.
IV. Weinlaube. 1903. 521.
V. Landw. Jahrbücher. 1901, p. 72.
VI. Bericht d. Kgl. Württ. Weinbau-Versuchsanstalt Weinsberg. 1904, p. 72.
VII. Württ. Wochenbl. f. Landwirtschaft. 1901. 755.
VIII. Bericht d. Kgl. Württ. Weinbau-Versuchsanstalt Weinsberg. 1901–1903, p. 49.
IX. Anleitung z. mikrosk. Untersuchung u. Reinzüchtung d. häufigsten im Most u. Wein vorkommenden Pilze. Stuttgart. 1891, p. 41.
X. Bericht d. Kgl. Weinbau-Versuchsanstalt Weinsberg. 1904, pp. 53 and 69.
XI. Landw. Jahrbücher. 1901. XXX. 497.

Meissner, Roderich,
I. Studien ü. d. Einfluss d. Essigsäure u. Milchsäure auf die Hefen Saaz, Frohberg u. Logos in Saccharoselösung. Dis. Berlin. 1897.

Melsens,
I. Note sur la vitalité de la levure de bière.—C. R. 1870. LXX. 629.

Meltzer, S. J.,
I. Über die fundamentale Bedeutung der Erschütterung für die lebende Materie. —Zeitschrift für Biologie. 1894. XXX. 464.—C. f. B. 1894. XVI. 743.

Mendelsohn, Benno,
See Cohn u. Mendelsohn.

Mendoza, Anton,
I. Über einen neuen Micrococcus.—C. f. B. 1889. VI. 566.

Menge, Karl,
I. Über einen Micrococcus mit Eigenbewegung.—C. f. B. 1892. XII. 49.—K. J. III. 44.
II. Über rote Milch.—C. f. B. 1889. VI. 596.

Messea, Al.,
I. Contribuzione allo studio delle ciglia dei batterii e proposito di una classificazione.—Rivista d'Igiene e Sanità publica. 1890. I. No. 14.—C. f. B. 1891. IX. 106.

Metschnikoff, Elias,
I. Pasteuria ramosa, un représentant des bactéries à division longitudinales.—Ann. Inst. Past. 1888. II. 165.—C. f. B. 1888. IV. 17.
II. Contributions à l'étude du pléomorphisme des bactéries. — Ann. Inst. Past. 1889. III. 61.—C. f. B. 1889. V. 511.
III. Virchow's Archiv. 1884. XCVI. 177.

Meulemeester, Emile de,
I. Germ. Patent No. 105626, April 30, 1898.—Ch. C. 1900. I. 492.

Meusel, E.,
I. Über Nitritbildung durch Bakterien.—Tageblatt der Naturforscher-Versammlung zu Graz. 1875. 55.—Ch. C. 1875. 678.

Meyen,
I. Wiegmann's Archiv. 1838. IV. 100.

Meyer, Lothar,
I. Chemische Untersuchung der Thermen zu Landeck in der Grafschaft Glatz. —Journal f. prakt. Chemie. 1864. LXXXXI. 1.

Miciol,
I. Note sur les végétations qui se développent pendant la fermentation du tabac.—Mémorial des Manufactures de l'Etat. Tabacs. 1891. II. 182.—K. J. II. 221.

Miescher, F.,
I. Über die chemische Zusammensetzung der Eiterzellen.—Hoppe-Seyler's Mediz.-chem. Untersuchungen. 1871. Heft 4. 441.

Migula, W.,
I. Über den Zellinhalt von Bacillus oxalaticus Zopf.—Arbeiten a. d. bakteriolog. Inst. der Techn. Hochschule zu Karlsruhe. 1894. I. 139.—C. f. B. 2. Abt. 1895. I. 242.—K. J. V. 54.

II. Über ein neues System der Bakterien. —Arbeiten a. d. bakteriolog. Inst. d. Techn. Hochschule zu Karlsruhe. 1895. I. 235.—C. f. B. 2. Abt. 1895. I. 406. 1896. II. 307.

III. Die Artzahl der Bakterien bei der Beurteilung des Trinkwassers.—C. f. B. 1890. VIII. 353.

IV. Über sogenannte Kapselbildung bei Bakterien. — Deutsche tierärztliche Wochenschrift. 1896. No. 4 u. 5.—C. f. B. 2. Abt. 1896. II. 583.—K. J. VII. 24.

MILBURN,

I. C. f. B. 2. Abt. 1904. XIII. 268.

MILLER, A., and HYDE, F.,

I. The Application of Pure Yeast (Hansen) to High Fermentation.—Transactions of the North of England Institute of Technical Brewing. 1894. III.—K. J. V. 183.

MILLON, E.,

I. Théorie chimique de la nitrification.—C. R. 1860. LI. 548.

II. Faits nouveaux concernant les métamorphoses alcooliques. — C. R. 1863. LVII. 235. 1864. LIX. 144.

MINCK, F.,

I. Zur Frage über die Einwirkung der Röntgen'schen Strahlen auf Bakterien und ihre eventuelle therapeutische Verwendbarkeit. — Münchener mediz. Wochenschrift. 1896. XXXXIII. 202. —C. f. B. 2. Abt. 1896. XIX. 631.—K. J. VII. 53.

MINSSEN,

See TACKE, IMMENDORF, HESSENLAND, SCHÜTTE und MINSSEN.

MIQUEL, P.,

I. Manuel pratique d'Analyse bactériologique des eaux. Paris. 1891.

II. Monographie d'un bacille vivant au delà de 70 centigrades.—Annales de Micrographie. 1888. I. 3.—C. f. B. 1889. V. 282.

III. Les organismes vivants de l'atmosphère. 1883.

IV. Annuaire de l'observatoire de Montsouris. 1879.

V. De la présence dans l'air du ferment de l'urée.—Bulletin de la Soc. chim. de Paris. 1878. XXIX. 387. 1879. XXXI. 391.—Ch. C. 1879. 633.

VI. Etudes sur la fermentation ammoniacale et sur les ferments de l'urée.—Annales de Micrographie. 1889. I. 414. 1889–1890. II. 53, 145. 1890–1891. III. 275, 305. 1893. V. 161 *et seq.* 1895. VII. 49. 1896. VIII. 55. 1897. IX. 302 *et seq.*—K. J. I. 176; II. 259; IV. 285.

VII. Sur le ferment soluble de l'urée.—C. R. 1890. CXI. 397.—K. J. I. 176.

VIII. De la présence dans l'air du ferment alcoolique.—C. R. 1878. LXXXVII. 759.—Z. g. Br. 1879. II. 57.

IX. Dixième mémoire sur les poussières organisées de l'air et des eaux.—Mémoire de l'Observatoire de Montsouris pour 1888.—C. f. B. 1888. IV. 276.

MIRSCH u. EBERHARD,

I. German Patent No. 149432.

MIRSKY,

I. Sur quelques causes d'erreur dans la determination des Aspergillées parasites de l'homme. Nancy. 1903.

MITCHELL und REICHERT,

I. Über Schlangengifte.—Ch. C. 1887. 1152.

MITSCHERLICH, Eilhard,

I. Über die Zusammensetzung der Wand der Pflanzenzelle.—Monatsberichte d. K. Akad. der Wiss. zu Berlin. 1840. 102.

II. Über die Mykose den Zucker des Mutterkornes.—Monatsberichte, etc. 1857. 469.—Ann. d. Chemie u. Pharmacie. 1858. CVI. 15.

III. Über die Asche der Hefe.—Monatsber., etc. 1845. 236.

IV. Monatsber. d. Kgl. Akad. Wiss. Berlin. 1841, p. 392.

MITTELMEIER, H.,

See SCHEIBLER und MITTELMEIER.

MIX, Charles L.,

I. On a Kephir-like Yeast found in the United States.—Contr. from the Cryptog. Laborat. of Harvard University. Vol. XVI.—Proceedings of the American Acad. of Arts and Sciences. 1891. XXVI. 102.

MIYOSHI, Manabu,

I. Über Chemotropismus der Pilze.—Bot. Ztg. 1. Abt. 1894. LII. 1.—C. f. B. 1. Abt. 1894. XVI. 193.

II. Die Durchbohrung von Membranen durch Pilzfäden. — Jahrbuch f. wissensch. Botanik. 1895. XXVIII. 269. —C. f. B. 2. Abt. 1895. I. 824.

III. Jahrb. wiss. Bot. 1895. XXVIII. 227.

MOELLER, Hermann,

I. Bemerkungen zu Franks Mitteilungen über den Dimorphismus der Wurzelknöllchen der Erbse.—Ber. d. D. Bot. Ges. 1892. X. 242.—C. f. B. 1893. XIII. 500.—K. J. III. 201, 205.

II. Über den Zellkern und die Sporen der Hefe.—C. f. B. 1892. XII. 537.—Ch. C. 1893. I. 43.—K. J. III. 37.

III. Weitere Mitteilungen über den Zellkern und die Sporen der Hefe.—C. f. B. 1893. XIV. 358.—K. J. IV. 47.

IV. Neue Untersuchungen über den Zellkern und die Sporen der Hefen.—Ber. d. D. Bot. Ges. 1893. XI. 402.—K. J. IV. 48.

MOHL, Hugo von,

I. Blaue Färbung der vegetabilischen Zellmembran durch Jod. Flora. 1840.

MOHR, P.,
I. Über das Vorkommen von Pentosanen, resp. Pentosen im Biere.—W. f. Br. 1895. XII. 769.—Ch. C. 1895. II. 549.—K. J. VI. 169.
II. W. f. Br. 1886. III. 200.

MOLISCH, Hans,
I. Die Pflanze in ihren Beziehungen zum Eisen. Jena. 1892. G. Fischer.—Ch. C. 1892. II. 332.—K. J. III. 53.
II. Die mineralische Nahrung der niederen Pilze.—Sitzgsber. d. K. Akad. d. Wiss. zu Wien; math.-nat. Kl. 1894. CIII. 1. Abt. 554.—Ch. C. 1895. I. 494.—K. J. V. 72.
III. Bemerkung über den Nachweis von maskiertem Eisen.—Ber. d. D. Bot. Ges. 1893. XI. 73.—Ch. C. 1893. I. 857.
IV. Die Ernährung der Algen. I. u. II.—Sitzgsber. d. K. Akad. d. Wiss. zu Wien; math.-nat. Kl. 1895. CIV. 1. Abt. 783. 1896. CV. 1. Abt. 633.—Ch. C. 1896. II. 496.
V. Untersuchungen über das Erfrieren der Pflanzen. Jena. 1897.—Bot. Ztg. 2. Abt. 1897. LV. 281.

MOLLER, F. J.,
I. Über die Einwirkung des elektrischen Stromes auf Bakterien.—C. f. B. 2. Abt. 1897. III. 110.—Ch. C. 1897. I. 768.—K. J. VIII. 68.

MOLLERAU,
See NOCARD et MOLLERAU.

MOLLIARD et COUPIN,
I. C. R. 1903. CXXXVI. 1695.
II. Rev. génér. de Botanique. 1903. XV. 401.

MONTESANO, Giuseppe,
See FERMI und MONTESANO.

MONTI, Achille,
See LILIENFELD und MONTI.

MORCK, D.,
I. Über die Formen der Bakteroiden bei den einzelnen Species der Leguminosen.—Dissertation. Leipzig. 1892.—K. J. II. 207.

MOREAU,
I. Ann. Soc. Roy. Sci. Med. et Nat. de Bruxelles. 1904. XII.—W. f. Br. 1905. XXII. 37.

MORGEN, August,
See BEHREND und MORGEN.

MORIN et CLAUDON,
I. C. R. 1887. CIV. 1109.

MORIN, Edouard Charles,
I. Sur les bases extraites des liquides ayant subi la fermentation alcoolique.—C. R. 1888. CVI. 360.—Bot. Ztg. 1889. XXXXVII. 120.
II. Sur la composition chimique d'une eau-de-vie de vin de la Charente-Inférieure.—C. R. 1887. CV. 1019.
See also CLAUDON et MORIN.

MORINI,
I. Malpighia. 1888–1889. II. 224.

MORITZ, E. R.,
I. Z. g. Br. 1891. XIV. 199.

MORITZ, J.,
I. Zur Gärungsfrage.—Ber. d. D. Chem. Ges. 1874. VII. 156, 434.
II. Chem. Ztg. 1886. X. 322.
III. Landw. Jahrbücher. 1884. 929.

MORITZ und MORRIS,
I. Handbuch der Brauwissenschaft (A Text-book of the Science of Brewing).—German translation by W. Windisch. Berlin. 1893. Parey. 12*s*.

MORPURGO, Giulio, u. BRUNNER, Alfred,
I. Über die Anwendung der mikrobiologischen Reaktion zum Nachweise des Arsens in Teerfarbstoffen. — Österr. Chemiker-Zeitg. 1898. I. 167.—Ch. C. 1898. II. 505.

MORREN, Charles,
I. Recherches sur la rubéfaction des eaux.—Mém. de l'Académie de Bruxelles. 1841, p. 70.

MORRIS, G. Harris,
I. Tötung der Keime in der Würze während des Sudprozesses.—Z. g. Br. 1890. XIII. 124.—Ch. C. 1890. I. 844.
II. Bemerkungen über die Glukase.—Transactions of the Institute of Brewing. 1893. VI. 132.—Z. g. Br. 1893. XVI. 203.—Ch. C. 1893. I. 837.—K. J. IV. 282.
III. Chem. News. 1895. LXXI. 196.

MORRIS, G. H. (2),
I. Brewing Trade Review. 1895. 91.
II. Journ. Fed. Inst. Brewing. 1893. 350.
See also MACFADYEN, MORRIS u. ROWLAND, MORITZ u. MORRIS, BROWN u. MORRIS.

MORRIS, G. H., and WELLS, J. G.,
I. Fractional Fermentation: A Contribution to the Study of the Amyloïnes (Maltodextrines).—Transactions of the Institute of Brewing. 1892. V. 133.—K. J. III. 120.

MOSLER, F.,
I. Über blaue Milch und durch deren Genuss herbeigeführte Krankheiten.—Virchow's Archiv f. pathol. Anatomie. 1868. XXXXIII.
II. Mykologische Studien am Hühnerei.—*Ibid.* 1864. XXIX. 510.
III. Virchow's Archiv. 1864. XXIX. 510.

MOSSO, A.,
I. Ein Gift, welches sich im Blute der Mureniden vorfindet.—Rendiconti della Accad. dei Lincei. Roma. 1887. IV. 665, 673.—Ch. C. 1888. 1004.

MOUGINET, Charles,
I. Quelques bactéries des putréfactions. Thèse. Nancy. 1894.—C. f. B. 2. Abt. 1895. I. 186.

MOUSSETTE,
I. Observations sur la fermentation panaire.—C. R. 1883. LXXXXVI. 1865.—Ch. C. 1883. 549.

MUDIE,
I. Journ. f. Pharmacie. 1832. XVIII. 705.
MÜHLHÄUSER,
I. Über Spirillen. — Virchow's Archiv. 1884. LXXXXVII. 84.
MÜLLER, Alexander,
I. Über den gegenwärtigen Stand der Städtereinigungs- und Wasserbeschaffungsfrage für Berlin.—D. Landw. Vers.-Stat. 1873. XVI. 241.
MÜLLER, C.,
I. Chemisch-physikalische Beschreibung der Thermen von Baden in der Schweiz. Baden. 1870.
MÜLLER, Carl,
I. Kritische Untersuchungen über den Nachweis maskierten Eisens in der Pflanze und den angeblichen Eisengehalt des Kaliumhydroxydes.—Ber. d. D. Bot. Ges. 1893. XI. 252.—Ch. C. 1893. II. 155.
MÜLLER-THURGAU, Hermann,
I. Der Milchsäurestich in Trauben- und Obstweinen. — III. Jahresbericht der Deutsch-schweizerischen Versuchs-Station und Schule für Obst-, Wein- und Gartenbau in Wädensweil für 1892–93. Zürich. 1894. 90.—K. J. V. 195.
II. Neue Forschungsresultate auf dem Gebiet der Weingärung und deren Bedeutung für die Praxis.—Bericht über die Verhandlungen des XI. Deutschen Weinbau-Kongresses in Trier. Mainz. 1889. 80.
III. Über die Ergebnisse neuer Untersuchungen auf dem Gebiete der Weinbereitung.—Bericht des XII. D. Weinbau-Kongresses in Worms. Mainz. 1891. 128.—K. J. II. 148.
IV. Weitere Untersuchungen über die Physiologie der Hefe und die Bedeutung ausgewählter und reingezüchteter Heferassen für die Weingärung.—III. Jahresbericht, etc., in Wädensweil. 1892–93. 73. Weinbau- u. Weinhandel. 1894. No. 24.—K. J. V. 169.
V. Neuere Erfahrungen bei Anwendung der Reinhefen in der Weinbereitung.—Ber., etc., d. XIV. D. Weinbau-Kongresses in Neustadt a. d. Hardt. 1895. 30.
VI. Das Braun- oder Rahnwerden der Weine.—Schweizerische Zeitschrift f. Obst- und Weinbau. 1894. III. 8.
VII. Über das Verhalten von Stärke und Zucker in reifenden und trocknenden Tabaksblättern.—Landw. Jahrbücher. 1885. XIV. 485.
VIII. Veränderungen, welche das Obst beim Faulen erleidet.—III. Jahresbericht, etc., in Wädensweil. 61.—Zeitschrift f. Pflanzenkrankheiten. 1895. V. 249.—K. J. V. 75.
IX. Die Edelfäule der Trauben.—Landw. Jahrbücher. 1888. XVII. 83.—C. f. B. 1888. IV. 179.—Ch. C. 1888. p. 957.—Bericht, etc., d. X. D. Weinbau-Kongresses zu Freiburg i. Br. 1887. 50.
X. Züchtung von Heferassen für bestimmte Zwecke.—V. Jahresbericht, etc., in Wädensweil pro 1894–95. 72.
XI. Das Zusammenwirken verschiedener Heferassen bei der Weingärung.—*Ibid.* 76.
XII. Die Herstellung unvergorener und alkoholfreier Obst- u. Traubenweine. 4th Ed. 1897. Frauenfeld. J. Huber.
XIII. Die Hefe als Kulturpflanze in den Weinbergen.—Weinbau und Weinhandel. 1894. 428.—IV. Jahresbericht, etc., in Wädensweil pro 1893–94. 68.
XIV. Über den Einfluss der Temperatur auf Verlauf und Produkt der Weingärung.—Ber. ü. d. Gen.-Vers. d. D. Weinbau-Vereines. 1884. 50.
XV. Botrytis und Peronospora als Schädiger der Gescheine und jungen Früchte des Weinstockes.—Weinbau und Weinhandel. 1888. VI. 256.
XVI. Einfluss der zugespitzten Hefe (Saccharomyces apiculatus) auf die Gärung der Obst- und Traubenweine.—VII. Jahresbericht, etc., in Wädensweil pro 1898. VII. 50.—C. f. B. 2. Abt. 1899. V. 684.—Ch. C. 1899. II. 916.—K. J. X. 148.
XVII. Einfluss der schwefligen Säure auf die Gärung.—VII. Jahresbericht, etc., in Wädensweil pro 1898. VII. 56.—C. f. B. 2. Abt. 1899. V. 788.—K. J. X. 128.
XVIII. In welcher Weise lässt sich die Weingärung günstig beeinflussen ?—Bericht, etc., des IX. D. Weinbau-Kongresses zu Rüdesheim. 1886. 66.
XIX. Welches ist die geeignetste Temperatur für die Weingärung.—Bericht, etc., des X. D. Weinbau-Kongresses zu Freiburg i. B. 1888. 94.
XX. Gewinnung und Vermehrung von Weinheferassen. — IV. Jahresbericht, etc., in Wädensweil pro 1893–94. p. 64. —K. J. VI. 182.
XXI. Ber. ü. d. Verhandl. d. VIII. D. Weinbau-Kongresses in Colmar, 1885. Mainz. 1886. 126.
XXII. Ber. ü. d. Verhandl. d. XI. D. Weinbau-Kongresses in Trier. Mainz. 1889, 87.
XXIII. Ber. ü. d. Verhandl. d. XII. D. Weinbau-Kongresses in Worms. Mainz. 1891, 129.
XXIV. Jahresbericht, etc., in Wädensweil pro 1896–97. VII. 56.—IX. Jahresber., etc., pro 1898–99, 74.
XXV. Die Verstellung unvergorener u. alkoholfreier Obst- u. Traubenweine. 3rd Ed. Frauenfeld. 1896.
XXVI. Ber. ü. d. Verhandl. d. XI. D. Weinbau-Kongresses in Trier. 1889, 88.
XXIX. Weinbau u. Weinhandel. 1889. Nos. 40 and 41.
XXX. V. Jahresber. d. Schweiz. Versuchsanstalt in Wädensweil f. 1894–95. 76.—Weinbau u. Weinhandel. 1897. No. 17.
See also CZÉH u. MÜLLER-THURGAU.

MÜNTZ, A.,
I. Sur la décomposition des roches et la formation de la terre arable.—C. R. 1890. CXI. 1370.—K. J. I. 109.
See also SCHLÖSING et MÜNTZ.

MÜNTZ, A., et GIRARD, A. Ch.,
I. Les pertes d'azote dans les fumiers.—C. R. 1893. CXV. 1318; CXVI. 108.—Ch. C. 1893. I. 270, 577.

MÜNTZ, A., et MARCANO, V.,
I. Sur la proportion des nitrates contenus dans les pluies des régions tropicales.—C. R. 1889. CVIII. 1062.—Ch. C. 1889. II. 156.

MULDER, Gerardus Johannes,
I. Sur quelques combinaisons de la protéine.—Bulletin des sciences physiques et naturelles en Néerlande. Rotterdam. 1838.—Journal f. prakt. Chemie. 1839. XVI. 297.
II. Die Chemie des Weines. — German trans. by Karl Arenz. 1856. Leipzig. J. J. Weber.
III. Untersuchung der Essigmutter.—Annalen d. Chemie und Pharmacie. 1843. XLVI. 207.
IV. Versuch einer allgemeinen physiologischen Chemie. German Ed. 1844–51. I. 203.
V. Het bier scheikundig beschouwd. Rotterdam. 1857.

MUNRO,
I. Über die Bildung und Zerstörung von Nitraten in künstlichen Lösungen und im Fluss- und Quellwasser.—Journal of the Chemical Society. 1886. XLIX. 632.—Ch. C. 1886. 700.—Biedermann's Centralblatt f. Agrikulturchemie. 1888. XVII. 649.

MÜNZER,
I. Münch. med. Wochenschrift. 1903. L. 1949.

MURPHY, J.,
I. Some Aspects of the Pure Yeast Question.—Journal of the Federated Institutes of Brewing. 1899. V. 432.—K. J. X. 118.

MUSCULUS,
I. Sur le ferment de l'urée.—C. R. 1876. LXXXII. 333.—Ch. C. 1876. 247.

MUSSET, Franz,
I. Zum Nachweis von Mutterkorn im Mehl.—Pharmac. Centralhalle. 1899. XL. 353.—Ch. C. 1899. II. 149.

MUSSO, Giov.,
See MANETTI und MUSSO.

NÄGELI, Carl von,
I. Mechanisch-physiologische Theorie der Abstammungslehre. 1884. München.
II. Theorie der Gärung. 1879. München.
III. Über Gärung ausserhalb der Hefezellen.—Zeitschrift f. Biologie. 1882. XVIII. 543.—Z. g. Br. 1882. V. 485.
IV. Die Ernährung der niederen Pilze.—Sitzungsberichte der Bayr. Akademie. Mathem.-physik. Kl. 1879. IX. 395.—Ch. C. 1881. 45.
V. Verhandlungen d. 33 Versammlung D. Naturforscher und Ärzte zu Bonn. 1857.—Bot. Ztg. 1857. XV. 760.
VI. Die niederen Pilze in ihren Beziehungen zu den Infektionskrankheiten und der Gesundheitspflege. München. 1877.
VII. Verhalten der Zellhaut zum Jod.—Sitzgsber. d. Bayr. Akad. d. Wiss. 1863. 383.

NÄGELI, C. von, u. LOEW, O.,
I. Über die Fettbildung bei den niederen Pilzen. — Journal f. prakt. Chemie. 1880. XXI. 97.—Reprinted in Z. g. Br. 1880. III. 499.—Ch. C. 1880. 231.
II. Über die chemische Zusammensetzung der Hefe.—Sitzgsber. d. Kgl. Akademie d. Wiss. in München. 1878. VIII. 161.—Reproduced in Z. g. Br. 1878. I. 337; also in Journal. f. prakt. Chemie. 1878. CXXV. 403.
III. Sitz. d. Kgl. Akad. d. Wiss. München. 1878. VIII. 173.

NASSE,
I. A. f. Hygiene. 1875. XI. 138.
II. *Ibid.* 1877. XV. 471.

NASTUKOFF, A.,
I. Essais sur le pouvoir réducteur des levures pures ; moyen de le mesurer.—C. R. 1895. CXXI. 535.—Ch. C. 1895. II. 1049.—K. J. VI. 59.
II. Über die Sporenbildung der russischen Weinhefen.—C. f. B. 2. Abt. 1898. IV. 420.—K. J. IX. 34.

NATHAN, L.,
I. Fortschritte auf d. Gebiete der Fruchtweinbereitung. Stuttgart. 1893.—Abst. in K. J. 1893. IV. 160.
II. W. f. Br. 1903. XX. 397.

NAUMANN, O.,
I. Über den Gerbstoff der Pilze. Dissert. Erlangen. 1895.—Bot. Ztg. 2. Abt. 1896. LIV. 157.

NEEDHAM, John Tuberville,
I. New Microscopical Discoveries. London. 1745.

NEELSEN, F.,
I. Studium über die blaue Milch.—Cohn's Beiträge zur Biologie der Pflanzen. III. 187.

NEISSER,
I. Versuche über die Sporenbildung bei Xerosebacillen, Streptokokken und Choleraspirillen.—Z. f. Hygiene. 1888. IV. 268.—C. f. B. 1888. IV. 139.

NENCKI, Leon,
I. Recherches chimiques sur les microbes produisant l'inflammation des glandes mammaires des vaches et des chèvres laitières.—Archives de sciences biologiques publ. p. l'Institut imp. de médecine expérim. St. Pétersbourg. 1892. I. 25.—K. J. III. 179.

NENCKI, L., und FABIAN, A.,
I. O przetworach fermentowanych z mleka, a mianowicie o kumysie i kefirze. —Gazeta Lekarska. 1887. No. 3.—C. f. B. 1. Abt. 1887. II. 523.

NENCKI, Marcel von,
I. Über Mischkulturen.—C. f. B. 1892. XI. 225.—K. J. III. 55.
II. Die isomeren Milchsäuren als Erkennungsmittel einzelner Spaltpilzarten.—C. f. B. 1891. IX. 304.—Ch. C. 1891. I. 884.—K. J. II. 17.
III. Der chemische Mechanismus der Fäulnis.—Journal f. prakt. Chemie. 1878. CXXV. 105.—Ch. C. 1878. 294.
IV. Untersuchungen über die Zersetzung des Eiweisses durch anaerobe Spaltpilze.—Sitzungsber. d. k. Akademie d. Wiss. zu Wien. Monatshefte für Chemie. 1889. X. 506.—C. f. B. 1890. VII. 129.—Ch. C. 1889. II. 847.
V. Über das Verhalten der Gelatine, des Eiweisses, des Leucins, Tyrosins und Glykokolls bei der Fäulnis mit Pankreas. Bern. 1876. — Medizin. Centralblatt. 1876. XV. 297.—Ch. C. 1877. 374.
VI. Journ. f. prakt. Chem. 1883. XVII. 105.

NENCKI, M. v., u. SCHAFFER, F.,
I. Über die chemische Zusammensetzung der Fäulnisbakterien. — Journal für prakt. Chemie. 1879. CXXVIII. 443.
See also MACFADYEN, NENCKI u. SIEBER.

NENCKI, M. v., u. SIEBER, N.,
I. Über die Bildung der Paramilchsäure durch Gärung.—Monatshefte f. Chemie. 1889. X. 532.—C. f. B. 1890. VII. 130.—Ch. C. 1889. II. 849.
II. Zur Kenntnis der bei der Eiweissgärung auftretenden Gase.—Sitzungsber. d. K. Akademie d. Wiss. zu Wien. Monatshefte f. Chemie. 1889. X. 526.—C. f. B. 1890. VII. 131.—Ch. C. 1889. II. 848.

NESSLER, Julius,
I. Der Tabak. Mannheim. 1867.
II. Die Bereitung, Pflege und Untersuchung des Weines. 7th Ed. Stuttgart. 1898. 6*s*.
III. Die Ursachen des Krankwerdens der Weine.—Bericht ü. d. Verh. d. XIV. D. Weinbau-Kongresses in Neustadt a. d. Hardt. 1895. 57.

NESTLER, Anton,
I. Über einen in der Frucht von Lolium temulentum L. entdeckten Pilz.—Ber. d. D. Bot. Ges. 1898. XVI. 207.—C. f. B. 2. Abt. 1899. V. 365.

NEUBAUER, C.,
I. Studien über den Rotwein.—Annalen der Önologie. 1872. II. 1.—Ch. C. 1872. 778.

NEUMANN, Albert,
See KOSSEL und NEUMANN.

NEUMANN, J.,
I. Über die Konservierung der Milch durch Kaliumbichromat, Ammoniak und Ammoniakverbindungen.—Milchzeitung. 1893. XXII. 453. — Ch. C. 1893. II. 1109.—K. J. IV. 202.

NEUMANN, R.,
See LEHMANN und NEUMANN.

NEUMANN-WENDER,
I. Die Presshefe des Handels. — Zeitschrift f. Nahrungsmittel-Untersuchung, Hygiene u. Warenkunde. 1896. X. 153.—K. J. VII. 149.

NEUMAYER, Johann,
I. Untersuchungen über die Wirkungen der verschiedenen Hefearten, welche bei der Bereitung weingeistiger Getränke vorkommen, auf den tierischen und menschlichen Organismus. — Dissert. München. 1890. — Z. g. Br. 1890. XIII. 297.—C. f. B. 1. Abt. 1893. XIII. 611.—K. J. I. 34.

NEUMEISTER, Richard,
I. Bemerkungen zu Eduard Buchner's Mitteilungen über "Zymase."—Ber. d. D. Chem. Ges. 1897. XXX. 2963.—Ch. C. 1898. I. 394.—K. J. VIII. 284.
II. Über das Vorkommen und die Bedeutung eines eiweisslösenden Enzymes in jugendlichen Pflanzen.—Zeitschrift f. Biologie. 1893. XII. 447. — Ch. C. 1894. I. 828.

NEUVILLE,
I. Les ferments industriels de l'Extrême-Orient. Paris. 1902. 175.

NEWCOMBE,
I. Ann. of Botany. 1899. XIII. 49.—Bot. Centralbl. 1898. LXXIII. 105.

NEWLANDS, R., and LING, A. R.,
I. J. Fed. Inst. Brewing. 1901. VII. 181.

NIEDERHÄUSER, Emil,
I. Über italienische Weine.—Pharmaceutische Centralhalle. 1891. XXXII. 15.—Ch. C. 1891. I. 383.

NIEDERSTADT,
I. Über die Wirkung des Zentrifugierens auf die Verteilung der Bakterien in der Milch.—Zeitschrift f. Nahrungsmittel-Untersuchung u. Hygiene. 1893. VII. 3.—K. J. IV. 205.

NIELSEN, J. Chr.,
I. Sur le développement des spores du Sacch. membranæfaciens, du Sacch. Ludwigii et du Sacch. anomalus. — Compte rendu, etc., de Carlsberg. 1894. III. 176.—C. f. B. 2. Abt. 1895. I. 187.—K. J. V. 36.

NIEMANN, F.,
See JESERICH und NIEMANN.

NIEMILOWICZ,
See KRATSCHMER u. NIEMILOWICZ.

NIGGL, Max,
I. Das Indol ein Reagens auf verholzte Membranen. — Flora. 1881. XLIV. 545.

NISHIMURA, T.,
I. Bull. Coll. Agricult. Tokyo. III. 191.

NISHIMURA, Tojosaku,
I. Untersuchungen über die chemische Zusammensetzung eines Wasserbacillus. —A. f. Hygiene. 1893. XVIII. 318.—Ch. C. 1893. II. 1007.—K. J. IV. 71.

NISSEN,
I. Über das Verhalten der Kerne in den Milchdrüsen-Zellen bei der Absonderung.—Archiv f. mikroskop. Anatomie. 1886. XXVI.

NIVIÈRE, G., et HUBERT, A.,
I. Sur la gomme des vins.—Revue intern. des falsific. 1896. IX. 48.—Ch. C. 1896. I. 898.

NOBBE, Friedrich,
I. Versuche über Leguminosen-Knöllchen. —Tageblatt d. Vers. D. Naturf. u. Ärzte zu Bremen. 1890.—K. J. I. 132.

NOBBE, Fr., und HILTNER, L.,
I. Wodurch werden die knöllchenbesitzenden Leguminosen befähigt, den freien atmosphärischen Stickstoff für sich zu verwerten? — D. landw. Versuchs-Stationen. 1893. XXXXII. 459.—K. J. IV. 215.

NOBBE, F., HILTNER, L., und SCHMID, E.,
I. Versuche über die Biologie der Knöllchenbakterien der Leguminosen, insbesondere über die Frage der Arteinheit derselben. — D. landw. Versuchs-Stationen. 1894. XXXXV. 1.—C. f. B. 2. Abt. 1895. I. 199.—Ch. C. 1894. II. 705.—K. J. V. 251.

NOBBE, F., SCHMID, E., HILTNER, L., u. HOTTER, E.,
I. Die Stickstoffassimilation der Leguminosen.—D. Landw. Versuchs-Stationen. 1891. XXXIX. 327.—C. f. B. 1892. XII. 685.—K. J. II. 199.
II. Über die physiologische Bedeutung der Wurzelknöllchen von Elæagnus angustifolius.—D. Landw. Versuchs-Stationen. 1892. XXXXI. 138.—K. J. III. 207.

NOCARD, E., et MOLLEREAU,
I. Sur une mammite contagieuse des vaches laitières. — Ann. Inst. Past. 1887. I. No. 3.—C. f. B. 1. Abt. 1888. III. 15.

NOLL, Alfred,
I. Über die Bildung von Lävulinsäure aus Nucleinsäuren.—Z. f. physiolog. Chemie. 1898. XXV. 430.—Ch. C. 1898. II. 723.

NOMURA,
I. Bot. Magazine. Tokyo. 1897. XI. No. 123, p. 31.

NORDHAUSEN, M.,
I. Beiträge zur Biologie parasitärer Pilze. —Pringsheim's Jahrbücher f. w. Bot. 1898. XXXIII. 1.—C. f. B. 2. Abt. 1899. V. 527.

NORDTMEYER, H.,
I. Über Wasserfiltration durch Filter aus gebrannter Infusorienerde.—Z. f. Hygiene. 1891. X. 145.—C. f. B. IX. 644.—K. J. II. 22.

NUTTAL, George H. F., u. THIERFELDER, H.,
I. Tierisches Leben ohne Bakterien im Verdauungskanal. — Z. f. physiolog. Chemie. 1895. XXI. 109; 1896. XXII. 62.—Ch. C. 1896. I. 118; II. 50.

OBERDÖRFFER, H. J.,
I. Über die Einwirkung des Ozons auf Bakterien. Dissertation. Bonn. 1889.—C. f. B. 1890. VII. 350.

OBERMAYER, F., und KERRY, R.,
I. Studien zur Kenntnis der Eiweissfäulnis. —Centralblatt f. Physiologie. 1894. VII. 806.—Ch. C. 1894. I. 825.—K. J. V. 74.

OBERMÜLLER, Kuno,
I. Über Tuberkelbacillenfunde in der Marktmilch. — Hygien. Rundschau. 1895. V. 878.—Ch. C. 1896. I. 126.

OBRASTZOW, S.,
See IWANOWSKI u. OBRASTZOW.

OGSTON,
I. Über Abscesse.—Arch. f. klin. Chirurgie. 1880. XXV.
II. Report on Micro-organisms in Surgical Diseases.—Brit. Med. Journ. 1881, p. 369.

OHLMÜLLER,
I. Über die Wirkung des Ozons auf Bakterien.—Arbeiten a. d. K. Gesundheits-Amte. 1892. VIII. 228.—C. f. B. 1892. XI. 773.—K. J. III. 72.

OKADA, K.,
I. Über einen (roten Farbstofferzeugenden) Bacillus.—C. f. B. 1892. XI. 1.—K. J. III. 43.

OKAMURA u. TAKAKUSU,
I. Quoted by KOZAI, II.

OLIVIER, L.,
See ETARD et OLIVIER.

OLTMANNS, Friedrich,
I. Über positiven und negativen Heliotropismus. — Flora. 1897. LXXXIII. Heft 1.—Bot. Ztg. 1897. LV. 23.

OMEIS, Th.,
I. Über den konzentrierten Traubenmost. —Forschungsberichte über Lebensmittel, Warenkunde und Hygiene. 1894. I. 474.—Ch. C. 1895. I. 183.

OMELIANSKY, V.,
I. Sur la fermentation de la cellulose.—C. R. 1895. CXXI. 653.—Ch. C. 1895. II. 1166.—Z. g. Br. 1896. XIX. 137.

ONO, N.,
I. Über die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize.—Journal of the College of Science, Imp. University of Tokyo. 1900. XIII. 141.—C. f. B. 2. Abt. 1902. IX. 154. —Ch. C. 1902. II. 1068.

Oppenheimer, Carl,
I. Die Fermente und ihre Wirkungen. Leipzig. 1900. 10*s*.—C. f. B. 2. Abt. 1900. VI. 706.
II. Die Fermente. 2nd Ed. 1903. 105.
III. *Ibid.* 2nd Ed. 1903, p. 242.

Oppenheimer,
I. Biologie der Milchkotbakterien des Säuglings.—C. f. B. 1889. VI. 586.

Ordonneau, Ch.,
I. Etude sur les produits volatils des vins et sur l'équation de fermentation des sucres par les Mycoderma cerevisiæ et ellipsoideus.—Bulletin de la Soc. chimique. 1886. XXXXV. 332. — C. R. 1886. CII. 217.—Z. g. Br. 1886. IX. 122.

Orlowski,
I. Wirkung des Arsens auf Wachstum u. chemische Zusammensetzung von Asp. niger. Dissert. St. Petersburg. 1902. (Russian.)—Bot. Centralbl. 1904. XCV. 302.

Ortloff, L.,
I. C. f. B. 2. Abt. 1900. VI. 676.

Osborne, A.,
I. Die Sporenbildung des Milzbrandbacillus auf Nährböden von verschiedenem Gehalt an Nährstoffen.—A. f. Hygiene. 1890. XI. 51.—K. J. I. 27.

Osborne, Thomas B., und Campbell, G. F.,
I. Die Nucleinsäure aus dem Embryo des Weizens und ihre Proteinverbindungen. —Journal of the American Chemical Society. 1900. XXII. 379.—Ch. C. 1900. II. 538.

Osborne, W. A.,
I. Z. f. physiolog. Chemie. 1899. XXVIII. 399.

Oser, Johann,
I. Untersuchungen über die Alkoholgärung.—Sitzgsber. d. Kais. Akademie der Wissenschaften Wien. Mathem.-naturw. Klasse. 1867. LVI. 2. Abt. 489.—Ch. C. 1869. I. 141.

Ost,
I. Chem. Ztg. 1896. XX. 762.
II. Ber. d. D. Chem. Ges. 1890. XXIII. 3006.

Ostertag, Robert,
I. Handbuch der Fleischbeschau. 3rd Ed. Stuttgart. 1899. 20*s*.

Osterwalder, A.,
I. Landw. Jahr. d. Schweiz. 1902. XVI. 498.
II. Weinbau u. Weinhandel. 1903. V. 169.

Osthoff,
I. Anlagen für die Versorgung der Städte mit Lebensmitteln. Markthallen, Schlachthöfe und Viehmärkte.—Weyl, Handbuch der Hygiene. 5. Bd. Jena. Gustav Fischer. 1892.—C. f. B. 1894. XVI. 718.

O'Sullivan, Cornelius,
I. Engl. Patent No. 19161, 1897.
II. Trans. Fed. Inst. Brewing. 1893. VI. 67.

O'Sullivan and Tompson,
I. Journ. Chem. Soc. 1890. LVII. 834.

Otto, Richard,
I. Beobachtungen und Ergebnisse bei der Untersuchung und Vergärung von Heidelbeermosten.—Landw. Jahrbücher. 1898. XXVII. 261.—C. f. B. 2. Abt. 1897. III. 428.—Ch. C. 1898. II. 734.—K. J. VIII. 123.

Oudemans,
I. Nederl. Kruidk. Arch. 1886. 2nd Series. IV. 535.
II. Arch. Néerlandaises des Sciences exactes et Natur. 1902. 2nd Series. VII. 288.
III. *Ibid.* 283.
IV. Nederl. Kruidk. Arch. 1904. 2nd Series. II. Suppl. 4, p 1123.

Overbeck, A.,
I. Zur Kenntnis der Fettfarbstoffproduktion bei Spaltpilzen.—Nova Acta Academiæ Cæsareæ Leopoldino-Carolinæ Germanicæ Naturæ Curiosorum. 1891. LV. 399.—K. J. II. 85.

Overbeck, O.,
I. Über eine neue Methode zur Entdeckung freien Schwefels im Hopfen.—The Brewer's Journal. 1891. No. 307.—K. J. II. 142.

Overbeck, Otto Gerhard Christoph,
I. German Patent No. 107737, Dec. 21, 1898.—Ch. C. 1900. I. 1008.

Overton, E.,
I. Über die osmotischen Eigenschaften der lebenden Pflanzen- und Tierzelle.—Vierteljahrschrift d. naturf. Ges. in Zürich. 1895.—Ch. C. 1895. II. 726.—Z. g. Br. 1896. XIX. 47.

Pabst,
I. Neues Ferment zur Bereitung billigen moussierenden Getränkes aus Zuckerlösung.—Soc. industr. de Mulhouse. 1890. K. J. I. 143.

Pässler, Johannes,
I. Gerbt Tannin die tierische Haut?—Chemiker-Zeitung. 1894. XVIII. 363. —Ch. C. 1894. I. 802.

Paetow, U.,
See Herzfeld und Paetow.

Pagès, C.,
See Arthus et Pagès.

Palladin, W. J.,
I. C. f. B. 2. Abt. 1904. XIII. 353.

Pallas,
I. Sammlung historischer Nachrichten über die mongolischen Völkerschaften. St. Petersburg. 1776. I. 133.

Palm, R.,
I. Über den chemischen Charakter des violetten Farbstoffes im Mutterkorn, sowie dessen Nachweis im Mehle.—Zeitschr. f. analyt. Chemie. 1883. XXII. 319.

PAMMEL, L. H.,
I. Some Bacteriological Work in the Dairy. Extracts from the Iowa Agricult. Experim. Stat. Bull. No. 21, p. 6.—C. f. B. 1894. XVI. 128.
II. An Aromatic Bacillus of Cheese (Bacillus aromaticus). Extracts, etc., p. 1.—C. f. B. 1894. XVI. 128.

PAMMEL, WEEMS, and LAWSON-SCRIBNER,
I. Iowa Geological Survey. 1901.

PANTANELLI,
I. Jahrb. wiss. Bot. 1904. XL. 303.

PANUM, P. L.,
I. Das putride Gift, die Bakterien, die putride Infektion oder Intoxikation und die Septikämie.—Virchow's Archiv. 1874. LX. 328.—Bibliothek for Läger. 1856. 253.

PASTEUR, Louis,
I. Mémoire sur les corpuscules organisés qui existent dans l'atmosphère.—Annales de Chim. et de Phys. 1862. LXIV. 6.—German transl. by von A. Wieler. Leipzig. Engelmann. 1892. 1*s.* 10*d.*
II. Influence de l'oxygène sur le développement de la levure et sur la fermentation alcoolique.—C. R. 1861. LII. 1260. 1863. LVII. 936.
III. Etudes sur la bière, ses maladies, causes qui les provoquent, procédé pour la rendre inaltérable, avec une théorie nouvelle de la fermentation. Paris. 1876. Gauthier-Villars.
IV. Faits nouveaux pour servir à la connaissance de la théorie des fermentations proprements dites.—C. R. 1872. LXXV. 784.
V. Note sur la production de l'alcool par les fruits.—C. R. 1872. LXXV. 1054.
VI. Etudes sur la maladie des vers à soie. 1870. I. 228.
VII. Mémoire sur la fermentation alcoolique.—Annales de Chimie et de Physique. 1860. 3ème série. LVIII. 323.—German transl. by Victor Griessmayer: Die Alkoholgärung. Augsburg. 1871.
VIII. Animalcules infusoires vivant sans gaz oxygène libre et déterminant des fermentations.—C. R. 1861. LII. 344 et 1260.—Journal f. prakt. Chemie. 1861. LXXXIII. 374.—Ch. C. 1864. 459.
IX. Nouvel exemple de fermentation déterminée par des animalcules infusoires pouvant vivre sans oxygène libre et en dehors de tout contact avec l'air de l'atmosphère. Fermentation du tartrate de chaux.—C. R. 1863. LVI. 416.—Journal f. prakt Chemie. 1863. LXXXIX. 351.—Ch. C. 1864. 460.
X. Mémoire sur la fermentation lactique.—C. R. 1857. XXXXV. 913. 1858. XXXXVII. 224. 1859. XXXXVIII. 337, 1149.
XI. Über die Asymmetrie bei natürlich vorkommenden organischen Verbindungen. 1860.—German transl. by M. u. A. Ladenburg. Leipzig. Engelmann. 7*d.*—C. R. 1860. LI. 298.
XII. Etudes sur le vin, ses maladies, causes qui les provoquent, procédés nouveaux pour le conserver et pour le vieillir. Paris. 1866.
XIII. Mémoire sur la fermentation acétique.—Annales scientifiques de l'Ecole normale supérieure.—Paris. 1864.
XIV. Etudes sur le vinaigre. Paris. 1868.—German transl. by E. Borgmann ("Der Essig"). Braunschweig. 1878.
XV. Nouveau procédé pour la fabrication du vinaigre.—C. R. 1862. LV. 28.—Dingler's Journal. 1862. CLXV. 303. 1871. CCI. 67.
XVI. Des altérations spontanées ou maladies des vins, particulièrement dans le Jura.—Etudes sur les vins; deuxième partie.—C. R. 1864. LVIII. 142.
XVII. Observations relatives à une note de M. Duclaux sur la germination dans un sol riche en matières organiques, mais exempt de microbes.—C. R. 1885. C. 66.—Ch. C. 1885. 142.
XVIII. Examen critique d'un écrit posthume de Claude Bernard sur la fermentation alcoolique. Paris. 1879.
XIX. Nouveaux faits concernant la fermentation alcoolique.—C. R. 1859. XXXXVIII. 640.
XX. Procédé pratique de conservation et d'amélioration des vins.—C. R. 1865. LX. 899; LXI. 274, 865, 979. 1869. LXIX. 577, 645, 905, 973.
XXI. Mémoire sur la fermentation de l'acide tartrique.—C. R. 1858. XXXXVI. 615.
XXII. Note relative au Penicillium glaucum et à la dissymétrie moléculaire des produits organiques naturels.—C. R. 1860. LI. 298.
XXIII. C. R. 1858. XLVII. 1011.
XXIV. Ann. de Chimie et de Phys. 1872. 4e Sèrie. XXV. 145.
XXV. Etudes sur la bière. 1876. 100.
XXVI. *Ibid.*, p. 73.
XXVII. *Ibid.*, p. 76.
XXVIII. *Ibid.*, p. 148.
XXIX. Cited by ROUX, III.
XXX. Etudes sur la bière. 1876. 150, 155, 176–178.
XXXI. Ann. de Chimie et de Phys. 1860. (3.) LVIII. 324.
XXXII. Die Alkoholgärung. — German transl. by Griessmayer. 1887. 27.
XXXIII. Etudes sur les vins. 636.
XXXIV. Ann. de Chimie et de Phys. 1860. (3.) LVIII. 323, 401.

PASTEUR, L., et JOUBERT,
I. Sur les germes des bactéries en suspension dans l'atmosphère et dans les eaux.—C. R. 1877. LXXXIV. 206.—Ch. C. 1877. 214.
II. Sur la fermentation de l'urée.—C. R. 1876. LXXXIII. 1.

PASTEUR, JOUBERT, et CHAMBERLAND,
I. La théorie des germes et ses applications à la Médecine et à la chirurgie.—C. R. 1878. LXXXVI. 1037.

PATOUILLARD et DELACROIX,
I. Bull. Soc. Mycol. de France. 1891. VII. 118.

PAUL, Theodor, und KRÖNIG, B.,
I. Über das Verhalten der Bakterien zu chemischen Reagenzien.—Zeitschrift f. physikal. Chemie. 1897. XXI. 414.—Bot. Ztg. 2. Abt. 1897. LV. 145.—Ch. C. 1897. I. 327.
II. Die gesetzmässigen Beziehungen zwischen Lösungszustand und Wirkungswert der Desinfektionsmittel.—Münch. medic. Wochenschrift. 1897. XXXXIV. 304.—C. f. B. I. Abt. 1897. XXI. 710.
See also KRÖNIG und PAUL.

PAUTZ und VOGEL,
I. Z. f. Biol. 1895. XXXII. 304.

PAYEN, Anselme,
I. Extrait d'un Rapport adressé à M. le Ministre de la Guerre sur une altération extraordinaire du pain de munition.—Annales de Chimie et de Physique. 1843. 3e sér. IX. 5.
II. Mémoire sur le développement des végétaux.—Mémoires présentés à l'Académie des sciences de France. 1846. IX. 21.
III. C. R. 1859. XLIX. 521.

PAYEN, Anselme, et PERSOZ, J. F.,
I. Mémoire sur la diastase, les principaux produits de ses réactions, et leurs applications aux arts industriels.—Ann. de Chimie et de Physique. 1833. LIII. 73.

PEDERSEN, Rasmus,
I. Sur l'influence que l'introduction de l'air atmosphérique dans le moût qui fermente exerce sur la fermentation.—Compte rendu, etc., de Carlsberg. 1878. I. 38.
II. Recherches sur quelques facteurs qui ont de l'influence sur la propagation de la levure basse du Saccharomyces cerevisiæ.—Compte rendu, etc., de Carlsberg. 1878. I. 22.

PEDLER,
I. Liebig's Ann. 1868. CXLVII. 243.

PEETERS,
I. Belg. Patent No. 131555 of 1897.—Engl. Patents Nos. 26985 of 1897 and 17278 of 1898.—Ch. C. 1898. I. 964.—K. J. IX. 103.

PEGLION, Victor,
I. Etudes sur la pourriture des raisins causée par le Botrytis cinerea.—Revue intern. de viticulture et d'œnologie. 1895, p. 414.—Zeitschr. f. Pflanzenkrankh. 1896. VI. 102.
II. Staz. sperim. agrarie ital. 1895. XXVIII. 369.
III. Rendic. della R. Accad. dei Lincei. 1897.—C. f. B. 2. Abt. 1901. VII. 745.

PELLET,
I. Chem. Zeit. 1900. XXIV. 356 (notice).

PELOUZE u. LIEBIG,
I. Liebig's Ann. 1836. XIX. 241.

PELOUZE, Théophile Jules, et GAY-LUSSAC, L. J.,
I. Sur l'acide lactique.—Annales de Chimie et de Physique. 1833. LII. 410.

PELOUZE, Théophile Jules, et GÉLIS, A.,
I. Sur l'acide butyrique.—C. R. 1843. XVI. 1262.—Journal f. prakt. Chemie. 1843. XXIX. 453.

PENZO, Rudolf,
I. Beitrag zum Studium der biologischen Verhältnisse des Bacillus des malignen Ödems.—C. f. B. 1891. X. 822.

PERDRIX, L.,
I. Sur les fermentations produites par un microbe anaérobie de l'eau.—Ann. Inst. Past. 1891. V. 286.—Ch. C. 1891. II. 252.—K. J. II. 240.

PÉRÉ, A.,
I. Sur la formation des acides lactiques isomériques par l'action des microbes sur les substances hydrocarbonées.—Ann. Inst. Past. 1893. VII. 737.—C. f. B. 1894. XVI. 121.—Ch. C. 1894. I. 411.—K. J. IV. 187.

PEREIRE u. GUIGNARD,
I. German Patent No. 139387.—Z. f. Spiritusind. 1903. XXVI. 131.

PERNOSSI, Leone,
See FERMI und PERNOSSI.

PERREY, A.,
See HAUTEFEUILLE et PERREY.

PERRONCITO, C., e MAGGIORA, A.,
I. Studien über bitteren Rotwein.—Weinlaube. 1884. 459.

PERSOON, Christian Hendrik,
I. Mycologia europæa. Erlangen. 1822. Sectio prima, p. 96.

PERSOZ,
I. Ann. de Chimie et de Phy[illegible]. 1834. LVI. 361.

PERSOZ, Jean François,
See PAYEN et PERSOZ.

PERTY, Maximilian,
I. Zur Kenntnis kleinster Lebensformen. Bern. 1852.

PETERS, W. L.,
I. Die Organismen des Sauerteiges und ihre Bedeutung für die Brotgärung.—Bot. Ztg. 1889. XXXXVII. 405. Gives antecedent bibliography.—C. f. B. 1889. VI. 228.—Ch. C. 1889. II. 847.

PETERSEN, Anton,
I. Sarcina im Biere ohne irgend eine Krankheitserscheinung.—Z. g. Br. 1890. XIII. 1.—C. f. B. 1890. VII. 606.—K. J. I. 71.
II. Einige Bemerkungen über das Lüften der Würze.—Z. g. Br. 1888. XI. 53.

PETIT, P.,
I. Sur les hydrates de carbone restant dans la bière.—C. R. 1897. CXXIV. 510.—Ch. C. 1897. I. 828.—K. J. VIII. 129.
II. Sur une différence entre les levures hautes et basses.—C. R. 1897. CXXIV. 93.—Ch. C. 1897. I. 423.—K. J. VIII. 97.
III. Sur l'oxydation des moûts de bière.—C. R. 1894. CXVIII. 1055.
IV. C. R. 1899. CXXVIII. 1176.

PETIT, P., et LABOURASSE, G.,
I. Sur la solubilisation des matières azotées du malt.—C. R. 1900. CXXXI. 349, 394.—Ch. C. 1900. II. 586, 680.

PETKOW,
I. Notice in Chem. Ztg. 1902. XXVI. 41.

PETRI, R. J.,
I. Zusammenfassender Bericht über Nachweis und Bestimmung der pflanzlichen Mikroorganismen der Luft.—C. f. B. 1887. II. 113.
II. Über die Widerstandsfähigkeit der Bakterien des Schweinerotlaufes in Reinkulturen und im Fleisch rotlaufkranker Schweine gegen Kochen, Schmoren, Braten, Salzen, Einpökeln und Räuchern. — Arbeiten a. d. Kaiserl. Gesundheitsamte. 1890. VI. 292.—C. f. B. 1890. VIII. 596.
III. Das Mikroskop. Von seinen Anfängen bis zur jetzigen Vervollkommnung für alle Freunde dieses Instruments. Berlin. 1896. R. Schoetz. 8*s*.

PETRI, R. J., und MAASSEN, A.,
I. Über die Bereitung der Nährbouillon für bakteriologische Zwecke.—Arbeiten a. d. Kais. Gesundheitsamte. 1892. VIII. 30.—C. f. B. 1892. XII. 484.—K. J. III. 15.
II. Über die Herstellung von Dauermilch, unter Anlehnung an Versuche mit einem bestimmten neueren Verfahren.—Arbeiten a. d. K. Gesundheitsamte. 1891. VII. Heft 1.—K. J. II. 193.
III. Weitere Beiträge zur Schwefelwasserstoffbildung aerober Bakterien und kurze Angaben über Merkaptanbildung derselben.—Arb. a. d. Kais. Gesundheitsamte. 1893. VIII. 490.—C. f. B. 1894. XV. 908.—K. J. IV. 88.
IV. Beiträge zur Biologie der krankheitserregenden Bakterien insbesondere über die Bildung von Schwefelwasserstoff durch dieselben unter vornehmlicher Berücksichtigung des Schweinerotlaufes. —Arbeiten a. d. Kais. Gesundheitsamte. 1892. VIII. 318.

PEUCH, F.,
I. Des effets de la salaison sur la virulence de la viande de porc charbonneux.—C. R. 1887. CV. 285.

PFEFFER, Wilhelm,
I. Über chemotaktische Bewegungen von Bakterien, Flagellaten und Volvocineen. —Untersuchungen a. d. Bot. Institut zu Tübingen. 1887. II. 582.—C. f. B. 1. Abt. 1888. III. 684.
II. Über Elektion organischer Nährstoffe. —Pringsheim's Jahrb. f. w. Bot. 1895. XXVIII. 205.—K. J. VI. 65.
III. Pflanzenphysiologie. 2nd Ed. 2 vols. Leipzig. 1897.
IV. Über lokomotorische Richtungsbewegungen durch chemische Reize.—Untersuchungen a. d. Bot. Institut in Tübingen. 1884. I. 363.—Ber. d. D. Bot. Ges. 1883. I. 532.—Bot. Ztg. 1884. XLII. 133.
V. Über die regulatorische Bildung von Diastase.—Sitzgsber. d. kgl. sächs. Ges. d. Wiss. 1896. Mathem.-physik. Klasse. 513.—C. f. B. 2. Abt. 1897. III. 425. —K. J. VII. 235.
VI. Jahrb. wiss. Bot 1895. XXVIII. 206.
VII. Verhandl. d. Ges. d. Naturf. u. Aerzte. 1899. II. 210.

PFEIFER, Fr.,
I. Über das Vorkommen von schwefliger Säure im Biere.—Z. g. Br. 1889. XII. 345.—Ch. C. 1889. II. 954.

PFEIFFER, Richard,
See FRAENKEL und PFEIFFER.

PFLÜGER, E.,
I. Die Bestimmung des Glycogens nach Brücke und Külz.—Pflüger's Archiv f. d. gesamte Physiologie. 1899. LXXV. 120.—Ch. C. 1899. I. 1167.
II. Eine neue Methode zur Bestimmung des Glycogens.—Pflüger's Archiv, etc. 1899. LXXVI. 531, 543.—Ch. C. 1899. II. 572, 573.

PFUHL, E.,
I. Über die Desinfektion der Typhus- und Cholera-Ausleerungen mit Kalk.—Z. f. Hygiene. 1889. VI. 97; VII. 363.—C. f. B. 1889. VI. 340. 1890. VII. 260. —Ch. C. 1889. I. 814. 1890. I. 411.
II. Weitere Fortschritte in der Flachsgewinnung.—A reprint from the Rigaer Industrie-Zeitung. 1895. Riga. N. Kymmel.—C. f. B. 1896. II. 275.

PHISALIX, C.,
See CHARRIN et PHISALIX.

PICHI, P.,
I. Sopra l'azione dei sali di rame nel mosto di uva sul Saccharomyces ellipsoideus. —Nuova Rass. di vitic. ed enol. Conegliano. 1891. Fasc. V.—C. f. B. 1892. XII. 662.—Ch. C. 1893. I. 184.
II. Annali della R. Scuola di Vitic. e di Enol. in Conegliano. 1892. I.

PICTET, Raoult, und WEYL, Th.,
I. Über die Herstellung von Dauermilch mit dem Apparate der Herren Neuhauss, Gronwald und Oehlmann. — Berliner klinische Wochenschrift. 1891. No. 41. —C. f. B. 1892. XII. 491.

PICTET, R., et YUNG, E.,
I. De l'action du froid sur les microbes. —C. R. 1884. XCVIII. 747.—Z. g. Br. 1884. VII. 193.

PIERRE u. PUCHOT,
I. Liebig's Ann. 1869. CLI. 299.

PINNER, A.,
See KRÄMER und PINNER.

PIORKOWSKI,
I. Über die Einwanderung des Typhusbacillus in das Hühnerei.—A. f. Hygiene. 1895. XXV. 145.—Ch. C. 1896. I. 266.

PIROTTA e RIBONI,
I. Studii sul latte. 1879. Pavia.
II. Rendiconti d. R. Istituto Lombardo. 1879.

PLAGGE,
I. Untersuchungen über Wasserfilter.—Veröffentlichungen a. d. Gebiete d. Militär-Sanitätswesens. Part 9. 1895.—Ch. C. 1896. I. 604.

PLAGGE und TRAPP,
I. Die Methoden der Fleischkonservierung.—Veröffentlichungen aus dem Gebiete des Militär-Sanitätswesens.—Part 5. Berlin. 1893.—C. f. B. 1893. XIII. 768.—K. J. IV. 105.—*See* TRAPP, A., I.

PLANITZ, Hans von der,
I. Das Bier und seine Bereitung einst und jetzt.—Z. g. Br. 1879. II. 13.

PLANTA, A. v., und SCHULZE, E.,
I. Ber. d. D. Chem. Ges. 1890. XXIII. 1692. 1891. XXIV. 2705.

PLATH, H.,
I. Über die Nitrifikation des Ammoniaks und seiner Salze.—Landw. Jahrbücher. 1887. XVI. 891.—Ch. C. 1888. 344.

PLAUCHU, E.,
I. Recherches sur la formation des eaux sulfureuses naturelles.—C. R. 1877. LXXXIV. 235.—Ch. C. 1877. 137.

PLAUT, Hugo,
I. Zur Konservierungstechnik.—C. f. B. 1889. V. 324.

PODWYSSOZKI, W.,
I. Kefyr, kaukasisches Gärungsferment und Getränk aus Kuhmilch. St. Petersburg. 1884.

POEHL, Alexander,
I. Zur Lehre von den Fäulnis-Alkaloiden.—Ber. d. D. Chem. Ges. 1883. XVI. 1975.

POGGIALE,
I. Sur une altération spéciale et extraordinaire du pain de munition.—Bulletin de l'Académie de médecine. 1871. XXXVI. 657.

POHL, C., und BAUER, H.,
I. Apparate für kontinuierliche Fortpflanzung reingezüchteter ober- und untergäriger Heferassen für Brauereien und Brennereien. German Patent No. 64372, March 17, 1892.—Z. g. Br. 1893. XVI. 3.

POHL, Josef,
I. Über Fischgifte.—Ch. C. 1894. I. 1061.

POLLACCI, Gino,
I. Sulla distribuzione del Fosforo nei tessuti vegetali.—Malpighia. 1894. VIII. 94.—Zeitschrift f. Pflanzenkrankheiten. 1895. V. 299.—Zeitschrift f. wissenschaftl. Mikroskopie. 1894. XI. 539.

POLZENIUSZ, F.,
See GODLEWSKI u. POLZENIUSZ.

POPOFF, D.,
I. Die Zeit der Erscheinung und die allmähliche Verbreitung der Mikroorganismen im Verdauungstraktus der Tiere.—Wratsch. 1891. No. 39.—C. f. B. I. Abt. 1892. XI. 214.—K. J. II. 63.

POPOFF, P. M.,
I. Über die Einwirkung von eiweissverdauenden Fermenten auf die Nucleinstoffe.—Z. f. physiolog. Chemie. 1894. XVIII. 533.—Ch. C. 1894. I. 289.

POPOFF,
I. Über Sumpfgasfäulnis.—Pflüger's Archiv f. d. ges. Physiologie. 1875. X. 113.—Dingler's Journal. CCXVI. 191.—Ch. C. 1875. 470.
II. Sur un bacille anaérobie de la fermentation panaire.—Ann. Inst. Past. 1890. IV. 674.—C. f. B. 1891. IX. 104.—Ch. C. 1891. I. 548.—K. J. I. 143.

POPP und BECKER,
I. Über die Verarbeitung erhitzter Milch in den Molkereien.—Hygien. Rundschau. 1893. III. 530.—K. J. IV. 201.

PORODKO,
I. Jahrb. wiss. Bot. 1904. XLI. 1.

POTTEVIN, H.,
I. Recherches sur le pouvoir antiseptique de l'aldéhyde formique.—Ann. Inst. Past. 1894. VIII. 796.—K. J. V. 93.
II. C. R. 1900. CXXXI. 1215.

POTTEVIN et NAPIAS,
I. C. R. Soc. Biol. 1898. (10.) V. 237.

POUCHET, A.,
I. Sur la germination des levures, des fermentations, et sur les végétaux qu'elles produisent.—C. R. 1868. LXVII. 376, 549.

POULSEN, S. V.,
See HOLM und POULSEN.

POWER, W. H.,
I. Milk-Scarlatina in London.—Report of the Medical Officer of the Local Government Board for 1885–1886. No. 8*a*, p. 73.—C. f. B. 1887. II. 217.

POZZI-ESCOT, Emm.,
I. Bull Soc. Chimique. 1901. XXVII. 557.
II. *Ibid.* 1902. (3.) XXVII. 346.
III. *Ibid.* XXVII. 460.
IV. *Ibid.* XXVII. 459.
V. *Ibid.* XXVII. 692.
VI. Bull. de l'Assoc. chim. de sucr. et distill. 1906. XXIII. 1021.
VII. C. R. 1903. CXXXVII. 495.
VIII. Bull. Soc. Chimique. 1902. (3.) XXVIII. 280.

PRANDTL, C.,
I. Dingler's Journ. 1865. CLXXVIII. 149.

Prazmowski, Adam,
I. Untersuchungen über die Entwicklungsgeschichte und Fermentwirkung einiger Bakterien-Arten.—Dissert. Leipzig. 1880.
II. Über Sporenbildung bei den Bakterien. —Biolog. Centralblatt. 1888. VIII. 301. C. f. B. 1888. IV. 325.
III. Über den genetischen Zusammenhang der Milzbrand- und Heubakterien.—Biolog. Centralblatt. 1884. IV. 393.
IV. Die Wurzelknöllchen der Erbse.—Vers.-Stat. 1890. XXXVII. 161; XXXVIII. 1.—C. f. B. 1889. V. 805. 1890. VIII. 379.—K. J. I. 112.

Prechtl, J. B.,
I. Zur Geschichte des bayrischen Bieres. —Z. g. Br. 1879. II. 469.

Preyer, A.,
I. D. Tropenpflanzer. 1901. V.—Abst. in C. f. B. 2. Abt. 1902. VIII. 715.

Preyss, M.,
I. Über den auffallenden Eiweissgehalt der ungarischen Weine als Ursache ihrer geringen Haltbarkeit und dessen Gegenmittel.—Weinbau. 1875. I. 189.

Pringsheim, H. H.,
I. Ber. d. D. Chem. Ges. 1905. XXXVIII. 486.

Prinsen-Geerligs, H. C.,
I. Einige chinesische Sojabohnenpräparate. —Chemiker-Zeitg. 1896. XX. 67.—Ch. C. 1896. I. 607.—K. J. VII. 250.
II. Eine technisch angewandte Zuckerbildung aus Reis.—Chemiker-Zeitg. 1895. XIX. 1681, 1805.—C. f. B. 2. Abt. 1896. II. 122.—Ch. C. 1895. II. 872, 1019. —K. J. VI. 315.
III. Ist Methylalkohol ein normaler Bestandteil in Rum und Arac?—Chemiker-Zeitg. 1898. XXII. 70.—Ch. C. 1898. I. 642.
IV. Ang-Khak, ein chinesischer Pilzfarbstoff zum Färben von Esswaren. —Chemiker-Zeitg. 1895. XIX. 1311. —C. f. B. 2. Abt. 1896. II. 234.—Ch. C. 1895. II. 469.—K. J. VI. 74.
V. Notice in Chem. Ztg. XXI. 150.
See also Went und Prinsen-Geerligs.

Prior, Eugen,
I. Über die Menge und Natur der beim Mälzen der Gerste, Darren und Maischen des Malzes und Kochen der Würze gebildeten Säuren. — Bayer. Brauerjournal. 1895. V. 181.—Z. g. Br. 1895. XVIII. 395.
II. Chemie und Physiologie des Malzes und Bieres. Leipzig. 1896. J. A. Barth.
III. Über die Erfahrungen beim Arbeiten mit Nürnberger Reinhefen. — Bayer. Brauerjournal. Nürnberg. 1891. I.
IV. Bayer. Brauerjournal. 1893. III. 2.
V. Chemie u. Physiologie d. Malzes u. d. Bieres. 1896. 397.
VI. Bayer. Brauerjournal. 1895. V. 49.
VII. *Ibid.* 1892. II. 1. 1894. IV. 469. 1895. V. 97.

Prior, E., und Schulze, H.,
I. Z. f. angew. Chemie. 1901. 208.

Prochownik und Spaeth,
I. Über die keimtötende Wirkung des galvanischen Stromes.—Deutsche medic. Wochenschrift. 1890. No. 26.—C. f. B. 1891. IX. 324.—K. J. I. 45.

Proskauer, Bernhard, u. Beck, M.,
I. Beiträge zur Ernährungsphysiologie des Tuberkelbacillus.—Z. f. Hygiene. 1894. XVIII. 128.—Ch. C. 1894. II. 995. —K. J. V. 74.
See also Fischer und Proskauer.

Proust, J. L.,
I. Recherches sur le principe qui assaisonne les fromages. — Journal de Physique. 1819. LXXXIX. 233.

Prove, Oskar,
I. Micrococcus ochroleucus, eine neue chromogene Spaltpilzform.—Cohn's Beiträge zur Biologie der Pflanzen. 1887. IV. 409.—C. f. B. 1887. II. 498.

Prudden, T. Mitchell,
I. On Bacteria in Ice and their Relations to Disease, with Special Reference to the Ice Supply of New York City. — New York Medical Record. 1887.—C. f. B. 1887. I. 650.

Purdie, T., and Walker, J. W.,
I. Resolution of Lactic Acid into its Optically Active Compounds.—Journal of the Chem. Soc. Transactions. 1892. LXI. 754.—Chem. News. 1892. LXVI. 352.—Ch. C. 1892. II. 352.—K. J. III. 171.
II. Darstellung der aktiven Milchsäuren und die Drehung von deren gelösten Metallsalzen.—Chem. News. 1895. LXXI. 278.—Ch. C. 1895. II. 157.

Puriewitsch, K.,
I. Über die Stickstoffassimilation bei den Schimmelpilzen.—Ber. d. D. Bot. Ges. 1895. XIII. 339.—Ch. C. 1896. I. 125.
II. Über die Wirkung des Lichtes auf den Atmungsprozess bei den Pflanzen.—Schriften d. Ges. d. Naturforscher in Kiew. 1890. XI. 211. (Russian.)
III. Über die Atmung der Schimmelpilze auf verschiedenen Nährlösungen.—Ber. d. D. Bot. Ges. 1898. XVI. 290.—C. f. B. 2. Abt. 1899. V. 223.—Ch. C. 1899. I. 1251.
IV. Schriften d. Ges. d. Naturforscher in Kiew. 1899. XVI.
V. Comptes rendus Soc. de Biologie. 1897. 686.
VI. Ber. d. D. Bot. Ges. 1898. XVI. 368.
VII. *Ibid.* XIII. 339.
VIII. *Ibid.* 1896. XIV. 210.

Quevenne, T. A.,
I. Sur la levure et la fermentation vineuse. —Journal de Pharmacie. 1838. XXIV. 265, 329.—Journal f. prakt. Chemie. 1838. XIV. 328, 458

QUINQUAUD, E.,
See GRÉHANT et QUINQUAUD, SCHUTZENBERGER et QUINQUAUD.

RABINOWITSCH, Lydia,
I. Über die thermophilen Bakterien.—Z. f. Hygiene. 1895. XX. 154.—C. f. B. 2. Abt. 1895. I. 585.—Ch. C. 1895. II. 165.

RABUTEAU,
I. Sur les effets des alcools de la série $C_nH_{2n+2}O$.—C. R. 1875. LXXXI. 631.
II. C. R. 1878. LXXXVII. 500.

RACIBORSKI, M.,
I. Mykologische Studien.—Anzeiger der Akademie d. Wissenschaften in Krakau. 1896. 377.
II. Cryptogamæ parasiticæ in insula Java lectæ exsiccatæ Buitenzorg. 1899. Fasc. II.
III. Bull. Acad. Sciences de Cracovie. Cl. des Sci. Math. et Nat. 1905. 693.

RADAIS, M.,
See SAUVAGEAU et RADAIS.

RADENHAUSEN, P.,
See DANILEWSKY und RADENHAUSEN.

RADZISZEWSKI, Bronislaus,
I. Über die Phosphorescenz der organischen und organisierten Körper.—Annalen d. Chemie. 1880. CCIII. 305.

RAPP, Rudolf,
I. Einfluss des Sauerstoffes auf gärende Hefe.—Ber. d. D. Chem. Ges. 1896. XXIX. 1983.—C. f. B. 2. Abt. 1896. II. 680.—Ch. C. 1896. II. 899.—K. J. VII. 86.
II. Münch. med. Woch. 1902. XLIX. 1494.
See also BUCHNER und RAPP.

RATHGEN, F.,
I. Über Konservierung antiker Bronzen.—Dingler's polyt. Journal. 1896. CCCI. 44.—Ch. C. 1896. II. 415.
II. Japans Volkwirthschaft u. Staatshaushalt. 1891.

RÁTZ, Stefan von,
I. Über die schleimige Milch.—Archiv f. Tierheilkunde. 1890. XVI. 100.—K. J. I. 87.

RAU, A.,
I. A. f. Hygiene. 1892. XIV. 225.

RAULIN, Jules,
I. Sur les conditions chimiques de la vie des organismes inférieurs.—C. R. 1870. LXX. 634.
II. Etudes chimiques sur la végétation des Mucédinées particulièrement de l'Ascophora nigrans.—C. R. 1863. LVII. 228.
III. Etudes chimiques sur la végétation.—Annales des sciences naturelles. Botanique. 5e série. 1869. XI. 93.

RAUM, Johannes,
I. Der gegenwärtige Stand unserer Kenntnisse über den Einfluss des Lichtes auf Bakterien und auf den tierischen Organismus.—Z. f. Hygiene. 1889. VI. 312.—C. f. B. 1889. VI. 261.
II. Zur Morphologie und Biologie der Sprosspilze.—Z. f. Hygiene. 1891. X. 1.—Z. g. Br. 1891. XIV. 273.—C. f. B. 1891. X. 79.—Ch. C. 1891. I. 882.—K. J. II. 38.
III. Z. f. Hygiene. 1891. X. 17.

RAUMER, E. v.,
I. Z. f. angew. Chemie. 1890. 421.

RAUX, G.,
I. Quoted by NOMURA, I.

RAVÀ, Jacopo,
I. L'acidità dell latte in rapporto alla Fabricazione dell Formaggio. Lodi. 1887. Constantino dell' Ava. — Milchzeitung. 1887. XVI. 422.

RAVAZ, L.,
I. Sur une maladie de la Vigne causée par le Botrytis cinerea.—C. R. 1894. CXVIII. 1289.—C. f. B. 2. Abt. 1895. I. 311.
II. La pourriture des raisins.—Revue de viticulture. 1895. IV. 183.
See also VIALA et RAVAZ.

RAVAZ, L., et GOUIRAND, G.,
I. Recherche sur le traitement de quelques maladies de la vigne. I. Pourriture grise (Botrytis cinerea).—Revue de viticulture. 1896. VI. 101.

RAY, Julien,
I. Sur le développement d'un champignon dans un liquide en mouvement.—C. R. 1896. CXXIII. 907.—Bot. Ztg. 2. Abt. 1897. LV. 68.

RAÝMAN, Bohuslav, und KRUIS, K.,
I. Chemisch-biologische Studien. — Mitteilungen der Versuchs-Station für Spiritusindustrie zu Prag. 1891. I.—C. f. B. 1. Abt. 1892. XII. 150.—Z. g. Br. 1892. XV. 94.—Ch. C. 1892. I. 211.—K. J. II. 125.
See also KRUIS und RAÝMAN.

RECHTER, de, und LEGROS,
I. Notiz über die Desinfektion durch Schwefeldioxyd und das Gasgemisch Pictet.—Ch. C. 1894. I. 827.—Reprinted from Presse méd. belge, 1893, No. 42, and Journal de Pharmacie et de Chimie, 1894, XXIX. 236.

REESS, Max.
I. Botanische Untersuchungen über die Alkoholgärungspilze. 1870. Leipzig. A. Felix.
II. Über die systematische Stellung der Hefepilze.—Bot. Ztg. 1884. XXXXII. 651.—Z. g. Br. 1884. VII. 442.
III. Zur Naturgeschichte der Bierhefe.—Bot. Ztg 1869. XXVII. 105.
IV. Botanische Untersuchungen ü. d. Alkoholgärungspilze. Leipzig. 1870. 71.

REGEL, E.,
I. Wirkung des Lichtes auf Pilze.—Sitzgsber. d. botan. Sect. d. St. Petersburger Naturforscher-Gesellschaft. 1881. — Bot. Ztg. 1882. XXXX. 29.

Regnard, P.,
I. Über Aufhaltung der Hefegärung durch Alkohole.—Comptes rendus de la Société de Biologie. 9e série. 1889. X. 124.—Centralblatt f. Physiologie. 1889. VI. 121.

Rehm, H.,
I. Die Pilze Deutschlands, Österreichs und der Schweiz. III. Abt.: Hysteriaceen und Discomyceten. Leipzig. 1896.

Reich,
I. Eine Unterleibstyphusepidemie infolge des Genusses ungekochter Molkereimilch.—Berliner klinische Wochenschrift. 1894. 702.—C. f. B. 1894. XVI. 704.

Reichard, Albert,
I. Studien über einen Sarcinaorganismus des Bieres.—Z. g. Br. 1894. XVII. 257.—K. J. IV. 197.
II. Über Blasengärung.—Z. g. Br. 1892. XV. 215.
III. Über Schaumbildung.—Z. g. Br. 1897. XX. 411.—K. J. VIII. 127.

Reichard, A., und Riehl, A.,
I. Zur Kenntnis und zur Bekämpfung der Sarcina-Krankheit.—Z. g. Br. 1895. XVIII. 59.—C. f. B. 2. Abt. 1895. I. 641.—K. J. VI. 201.
II. Versuche über Einwirkung der Hefegabe auf das Bier.—Z. g. Br. 1897. XX. 8.—K. J. VIII. 125.

Reichardt,
I. Verhandl. d. Zool. bot. Ges. in Wien. 1867. XVII. 335.

Reichert, Edward,
See Mitchell und Reichert.

Reimers, J.,
I. Über den Gehalt des Bodens an Bakterien. Dissert. Jena. 1889. Gives the antecedent bibliography.—C. f. B. 1. Abt. 1891. X. 489.

Rein,
I. Japan. 1886.

Reinhardt, M. O.,
I. Das Wachstum der Pilzhyphen.—Pringsheim's Jahr. f. wiss. Bot. 1892. XXIII. 479.

Reinke, J.,
I. Über den Einfluss mechanischer Erschütterung auf die Entwicklung der Spaltpilze.—Pflüger's Archiv. 1880. XXIII. 434.

Reinke, Otto,
I. Die Klärung der Biere durch Licht.—W. f. Br. 1896. XIII. 400.—K. J. VII. 113.
II. Über den Vergärungsgrad. Vortrag.—W. f. Br. 1889. VI. 569.
III. Die Konservierung von Hefen.—W. f. Br. 1888. V. 745. 1891. VIII. 703. 1892. IX. 1009.—K. J. III. 162.
IV. W. f. Br. 1891. VIII. 809.

Reinmann, R.,
I. C. f. B. 2. Abt. 1900. VI. 131.

Reinsch, A.,
I. Die Bakteriologie im Dienste der Sandfiltrationstechnik.—C. f. B. 1894. XVI. 881.—K. J. V. 24.
II. Zur bakteriologischen Untersuchung des Trinkwassers.—C. f. B. 1891. X. 415.

Reiset, Jules,
I. Expériences sur la putréfaction et sur la formation des fumiers.—C. R. 1856. XXXXII. 53. 1889. CVIII. 708, 779.—Ch. C. 1889. I. 846.
II. Note sur la production du gaz nitreux pendant la marche des fermentations dans les distilleries.—C. R. 1868. LXVI. 177.—Ch. C. 1868. 939.

Reiss, Rudolf,
I. Über die Natur der Reserve-cellulose und über ihre Auflösungsweise bei der Keimung der Samen.—Landwirtschaftl. Jahrbücher. 1889. XVIII. 711.—Ch. C. 1890. I. 165.

Renk,
I. Über Marktmilch in Halle.—Münchner medicinische Wochenschrift. 1891. No. 6 u. 7.—C. f. B. 1891. X. 193.

Renon,
I. Etude sur l'aspergillose chez les animaux et chez l'homme. Paris. 1867.

Rey-Pailhade, J. de,
I. Sur un corps d'origine organique hydrogénant le soufre à froid.—C. R. 1888. CVI. 1683.—Ch. C. 1888. 1085.
II. Nouvelles recherches physiologiques sur la substance organique hydrogénant le soufre à froid.—C. R. 1888. CVII. 43.—Ch. C. 1889. I. 439.
III. Sur de nouvelles propriétés chimiques de l'extrait alcoolique de levure de bière.—Bulletin de la Société chimique de Paris. Troisième série. 1890. III 171.—C. f. B. 1. Abt. 1890. VIII. 106.—Ch. C. 1890. I. 682.—K. J. I. 32.
IV. Action de l'alcool et du soufre sur la levure de bière.—Comptes rendus de la Soc. de Biologie. 1893. (10.) II. 46.—K. J. IV. 149.
V. Etudes sur les propriétés chimiques de l'extrait alcoolique de levure de bière: formation d'acide carbonique et absorption d'oxygène.—C. R. 1894. CXVIII. 201.—Ch. C. 1894. I. 472.—K. J. V. 114.
VI. Rôles respectifs du philothion et de la laccase dans les graines en germination.—C. R. 1895. CXXI. 1162.—Ch. C. 1896. I. 562.—K. J. VI. 331.
VII. Existence du corps protéique prévu par M. G. Bertrand dans la constitution des oxydases.—Bulletin de la Société chimique. 1897. (3.) XVII. 756.—Ch. C. 1897. II. 595.—K. J. VIII. 274.
VIII. Fermentation chimique par la levure en milieu antiseptique.—Bulletin de la Soc. chim. 1900. (3.) XXIII. 666.—Ch. C. 1900. II. 729.
IX. C. R. 1902. (3.) XXVII. 6.
X. *Ibid.* 1904. (3.) XXXI. 987.
XI. Comptes Rendus Soc. Biol. 1898. (10.) V. 372.
XII. Bull. Soc. chimique. 1906. (3.) XXXV. 1030.
XIII. *Ibid.* XXXV. 1030.

RIBONI,
See PIROTTA e RIBONI.

RICHARDS, Herbert Maule,
I. Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize.—Jahrbücher f. wissenschaftl. Botanik. 1897. XXX. 665.—Bot. Ztg. 2. Abt. 1897. LV. 377. —Z. g. Br. 1897. XX. 585.

RICHARDSON,
I. The Action of Light in preventing Putrefactive Decomposition and inducing the Formation of Hydrogen Peroxide in Organic Liquids.—Transactions of the Chemical Society. 1893. XXXXIII. 1109.—C. f. B. 1894. XVI. 42.—K. J. IV. 120.
II. The Action of Light on Oxalic Acid.—Chem. News. 1894. LXIX. 226.—Ch. C. 1894. I. 1144.

RICHARDSON, F. W.,
I. Journ. Federated Inst. Brewing. 1898. IV. 128.

RICHET, Ch.,
I. De l'action de quelques sels métalliques sur la fermentation lactique.—C. R. 1892. CXIV. 1494.—C. f. B. 1892. XII. 866. —K. J. III. 170.

RICHTER, A.,
I. Abst. in Botan. Centralbl. 1902. LXXXIX. 621.
II. C. f. B. 2. Abt. 1902. VIII. 787.

RICHTER, Andreas,
I. Zur Frage der chemischen Reizmittel.—C. f. B. 2. Abt. 1901. VII. 417.

RICHTER, Karl,
I. Beiträge zur genaueren Kenntnis der chemischen Beschaffenheit der Zellmembran bei den Pilzen. — Sitzungsber. d. K. Akademie d. W. zu Wien. 1881. LXXXIII. I. 494.—Ch. C. 1881. 483.

RIEHL, Albert,
See REICHARD und RIEHL.

RIETSCH, M.,
See MARTINAND et RIETSCH.

RIETSCH, M., et HERSELIN, M.,
I. Sur la fermentation apiculée et sur l'influence de l'aération dans les fermentations à température élevée.—Progrès agricole et viticole. 1895.—C. R. 1895. CXXI. 378.—Z. g. Br. 1896. XIX. 47. —Ch. C. 1895. II. 871.

RIJN, J. J. L. van,
I. Die Glykoside. Berlin. 1900. 10*s*.—C. f. B. 2. Abt. 1900. VI. 743.

RINDFLEISCH,
I. Untersuchungen über niedere Organismen. —Virchow's Archiv. 1873. LIV.

RINGEL, T.,
I. Über den Keimgehalt der Frauenmilch.—Münchner medic. Wochenschrift. 1893. XXXX. 513.—C. f. B. 1893. XIV. 429. —Ch. C. 1893. II. 695.

RINGER, Sydney,
I. Einwirkung von Kalksalzen auf Caseïn und Milch.—Journal of Physiology. 1891. XI. 464; XII. 164.—Ch. C. 1891. I. 702. 1892. I. 172.—K. J. II. 259; III. 261.

RIPPER, M.,
I. Weinbau u. Weinhandel. 1890. VIII. 168.
II. J. f. prakt. Chemie. 1892. XLVI. 428.
III. Forschungsberichte u. Lebensmittel. 1895. II. 12, 35.

RIPPER, Maximilian,
I. Die schweflige Säure im Weine und deren Bestimmung.—Ch. C. 1891. I. 46. 1893 I. 280. 1895. I. 566, 815.

RISCHLET und TOLLENS,
I. Ber. d. D. Chem. Ges. 1885. XVIII. 2611.

RISS,
I. Zeits. d. Landw. Vereins im Bayern. 1891. —Noticed in Koch's Jahresb. 1891. II. 170.

RIST, E., et KHOURY, J.,
I. Ann. Inst. Past. 1902. XVI. 65.

RITSERT, E.,
I. Untersuchungen über das Ranzigwerden der Fette. Bern. 1890.—Ch. C. 1890. II. 507.
II. Bakteriologische Untersuchungen über das Schleimigwerden der Infusa.—Pharm. Zeitung. 1891. XXXVI. 715, 774.—C. f. B. 1892. XI. 730.—Ch. C. 1892. I. 236.—K. J. II. 223; III. 59.

RITTHAUSEN, H.,
I. Chem. Ztg. 1897. XXI. 717.

RITTHAUSEN, H., und BAUMANN,
I. Über Zerstörung von Fett durch Schimmelpilze.—D. landw. Versuchs-Stationen. 1896. XXXXVII. 389.—C. f. B. 2. Abt. 1896. II. 711.—Ch. C. 1896. II. 680.—K. J. VII. 46.

ROBERTS, W.,
I. Studies on Biogenesis. — Philosophical Transactions of the Royal Society of London. 1874. CLXIV. II. 474.

ROBERTSON, J. Chalmers,
I. Symptoms of Irritant Poisoning in a Family, due to Diseased Bread. — The Lancet. 1887. II. 518.

ROBIN, Ch.,
I. Sur la nature des fermentations en tant que phénomènes nutritifs des assimilateurs des plantes.—Journal de l'anatomie et de physiologie. 1875, p. 379.
II. Des végét. qui croissent s. les anim. viv. 1847.

ROBIQUET,
I. Journ. de Pharm. et de Chim. 1852. XXII. 121.

ROCHARD, Felix,
I. Du parasitisme végétal dans les altérations du pain.—Annales d'Hygiène publique et de médecine legale. 1873. 2e série. XXXX. 83.

ROEDER, Georg,
See FISCHER, E., u. ROEDER., G.

ROESER, P.,
I. Contribution à l'étude de l'influence de la température sur les variations morphologiques et évolutives des micro-organismes. —Archives de médecine expérimentale et d'anatomie pathologique. 1890, p. 139.—C. f. B. VII. 476.—K. J. I. 39.
II. De la formation d'aldéhyde dans la fermentation alcoolique.—Ann. Inst. Past. 1893. VII. 41.—Ch. C. 1893. I. 837.—K. J. IV. 150.

ROESLER, L.,
I. Die Anwendung der schwefligen Säure in der Kellerwirtschaft und der Schwefelsäure-Gehalt des Weines.—Mitteilungen der chem.-phys. Vers.-Stat. zu Klosterneuburg bei Wien. 1885. IV. 9.

ROGER,
I. Fabrication des fromages de Brie, etc. Communication to the Société de l'Agriculture de Meaux. 1898.

ROGERS, L. A.,
I. C. f. B. 2. Abt. 1903. X. 381.
II. *Ibid.* 1904. XII. 338.

RÖHLING, A.,
I. Morphol. u. physiol. Untersuchungen ü. einige Rassen d. Sacch. apic. Dissert. Erlangen. 1905.

RÖHMANN, F.,
I. Nachtrag zur Arbeit von B. Bienstock: Über die Bakterien der Fäces.—Zeitschrift f. klin. Medicin. 1884. VIII. 43.—Ch. C. 1884. 646.

ROHN, Severin, u. WICHMANN, H.,
I. Notiz über einen bemerkenswerten Fall von unreinem Tiefbrunnenwasser. — Mitteilungen d. Österr. Versuchs-Station f. Brauerei u. Mälzerei in Wien. Heft II.—C. f. B. 1889. V. 642.—Ch. C. 1889. II. 93.

ROHRER,
I. Über die Pigmentbildung des Bacillus pyocyaneus.—C. f. B. 1892. XI. 327. —K. J. III. 86.

ROLANTS, E.,
See BOIDIN et ROLANTS.

ROLOFF,
I. Über die Milzbrand-Impfung und die Entwicklung der Milzbrand-Bakterien. —Archiv f. wissenschaftl. u. prakt. Tierheilkunde. 1883. IX. 459.

ROMMEL, W.,
See SITNIKOFF und ROMMEL.

ROMMEL, W., u. SITNIKOFF, A.,
I. Bull. de l'Ass. belge des Chimistes. 1903. XVIII. 1049.

ROMMIER, A.,
I. Sur la diminution de la puissance fermentescible de la levure ellipsoidale de vin, en présence des sels de cuivre.—C. R. 1890. CX. 536.—C. f. B. 1892. XII. 662.—Ch. C. 1890. I. 803.—K. J. I. 78.
II. C. R. 1890. CX. 1341.

ROOS, L.,
I. Die Mannitgärung der Weine.—Journal de pharmacie et de chimie. 1893. XXVII. 405. —Ch. C. 1893. I. 1098.—K. J. IV. 152.

RÖSE, B., und SCHULZE, E.,
I. Über einige Bestandteile des Emmenthaler Käses. — D. Landw. Versuchs-Stationen. 1885. XXXI. 115.

ROSENBACH,
I. Gibt es Spaltpilze oder deren Keime in den Geweben, im Blute, Lymphe gesunder Menschen und Tiere?—Deutsche Zeitschrift für Chirurgie. 1880. XIII. 344.
II. Mikroorganismen bei den Wundinfektionskrankheiten des Menschen. Wiesbaden. 1884.

ROSENSTIEHL, A.,
I. C. R. 1900. CXXX. 195. 1902. CXXXIV. 119.

RÖSSLER, Oskar,
I. Über die Kultivierung von Crenothrix polyspora auf festem Nährboden. — Archiv der Pharmacie. 1895. CCXXXIII. 189.—Ch. C. 1895. II. 173.

ROTH, O.,
I. Über das Vorkommen von Tuberkelbacillen in der Butter.—Korrespondenzblatt f. schweizer. Ärzte. 1894. No. 17. — Hygien. Rundschau. 1894. IV. 1132.—Ch. C. 1895. I. 279.

ROTHENBACH, F.,
I. Z. f. Spiritusind. 1896. XIX. 8.

ROTHENBACH, F., u. EBERLEIN, L.,
I. Die deutsche Essigindustrie. 1905. IX. 233.

ROTHERT, W.,
I. Über Sclerotium hydrophilum Sacc., einen sporenlosen Pilz.—Bot. Ztg. 1892. L. 321.

ROUX, E.,
I. De l'action de la lumière et de l'air sur les spores de la bactéridie du charbon.—Ann. Inst. Past. 1887. I. 445.—Ch. C. 1888. 554.
II. Bull. Soc. Chim. XXXV. 37.
III. Ann. de la Brasserie et de la Distillerie. Paris. 1898. I. 512.

ROWLAND, Sydney,
I. Cheese and Butter as Possible Carriers of Typhoid and Cholera Infection.—Brit. Medic. Journal. 1895. I. 1392.—C. f. B. 1. Abt. 1895. XVIII. 204.—Ch. C. 1895. II. 875.
See also HARDEN and ROWLAND; MACFADYEN, MORRIS and ROWLAND.

ROWLANDSON,
I. On the Production of Butter.—Journal of the Royal Agricult. Society of England. 1852. XIII. 35.

ROZE,
I. C. R. 1897. CXXV. 982.

ROZIER, François,
I. Cours complet d'agriculture, etc., ou Dictionnaire universel d'agriculture. 1786. IV. 525.

RÜBNER, M.,
I. Die Wanderungen des Schwefels im Stoffwechsel der Bakterien. — A. f. Hygiene. 1893. XVI. 78.—C. f. B. 1893. XIV. 64, 66.—K. J. IV. 95.
II. Uber das Vorkommen von Merkaptan. —A. f. Hygiene. 1893. XIX. 136.—Ch. C. 1894. I. 90.—K. J. IV. 97.
III. A. f. Hygiene. 1903. XLVIII. 260.
IV. *Ibid.* 1904. XLIX. 355.

RULLMANN, W.,
I. Chemisch - bakteriologische Untersuchungen von Zwischendeckenfüllungen mit besonderer Berücksichtigung von Cladothrix odorifera. — Forschungsber. über Lebensmittel, etc. 1895. II. 177.—C. f. B. 1. Abt. 1895. XVII. 884.—Ch. C. 1895. II. 372.
II. Weitere Mitteilungen über Cladothrix odorifera. — C. f. B. 2. Abt. 1896. II. 116.—K. J. VII. 58.

RUSSELL, H. L.,
I. The Effect of Mechanical Movement upon the Growth of Certain Lower Organisms. — The Botanical Gazette. 1892. No. 1. Bloomington, Indiana.
II. Untersuchungen über im Golf von Neapel lebende Bakterien. — Z. f. Hygiene. 1891. XI. With two plates. —K. J. II. 58.

RUSSELL, N. L.,
I. A Biological Study of Pasteurised Milk and Cream under Commercial Conditions.—C. f. B. 2. Abt. 1895. I. 741.

SAARE, Oskar,
I. Über Brennereibetrieb in Belgien und das Amyloverfahren.—Z. f. Spiritusind. 1900. XXIII. 419.

SACC,
I. De la panification aux Etats-Unis, et des propriétés du houblon comme ferment. — C. R. 1875. LXXXI. 1130. 1876. LXXXIII. 361.

SACCARDO, P.,
I. Sylloge fungorum omnium hucusque cognitorum. 16 vols. Patavii. 1882–1902.
II. *Ibid.* 1895. XI. 457.
III. Michelia. II. 547. — Fungi italici. Pl. 893. — Sylloge fungorum. 1886. IV. 85.
IV. Zusammenstellung der Penicillium spezies in Sylloge fungorum. IV. 78; X. 527; XI. 513; u. d. Aspergilleen in I. 25 (Eurotium); IV. 64; X. 524; XI. 591.

SACCARDO und SYDOW,
I. Sylloge fungorum. 1902. XVI. 818.

SADEBECK, R.,
I. Über Pythium Anguillulæ aceti.— Sitzungsbericht d. Ges. f. Botan. z. Hamburg. 1886. II. 39.—C. f. B. 1887. I. 50.
II. Untersuchungen über die Pilzgattung Exoascus und die durch dieselbe um Hamburg hervorgerufenen Baumkrankheiten.—Jahresbuch der wissenschaftl. Anstalten zu Hamburg für 1883.—Bot. Ztg. 1884. XXXXII. 655.
III. Kritische Untersuchungen über die durch Taphrina-Arten hervorgebrachten Baumkrankheiten. — *Ibid.* 1890. VIII.—C. f. B. 1. Abt. 1891. IX. 576.
IV. Die parasitischen Exoasceen.—Bot. Ztg. 1893. X.—C. f. B. 1. Abt. 1894. XV. 503.

SAIDA,
I. Ber. d. D. Bot. Ges. 1901. XIX. 107.

SAITO, K.,
I. Journ. of the College of Science, Imp. Univ. Tokyo. 1904. XIX. Art. 18, p. 1.
II. Botan. Mag. Tokyo. 1905. XIX. 75.
III. Journ. of the College of Science, Imp. Univ. Tokyo. 1904. XVIII. Art. 5.
IV. Bot. Mag. Tokyo. 1903. XVIII. 75.

SALFELD,
I. Ein Versuch mit Impferden verschiedener Herkunft auf Naturboden bei Pferdebohnen und Erbsen. — Deutsche landw. Presse. 1892. 648.

SALKOWSKI, E.,
I. Über die antiseptische Wirkung des Chloroformwassers.—Deutsche medicin. Wochenschrift. 1888. No. 16.—C. f. B. 1. Abt. 1888. IV. 188. 1889. V. 428.—Ch. C. 1888. 718. 1889. I. 613.
II. Über Zuckerbildung und andere Fermentationen in der Hefe.—Z. f. physiolog. Chemie. 1889. XIII. 506. —Ch. C. 1889. I. 591.
III. Über Autodigestion der Organe.— Z. f. klin. Medicin. 1890.—Ch. C. 1890. II. 524.
IV. Fermentative Prozesse in den Geweben.—Archiv d. Anat. u. Physiol., Physiol. Abt. 1890. 554. — Ch. C. 1891. I. 223.—K. J. II. 90.
V. Über die Kohlehydrate der Hefe. —Ber. d. D. Chem. Ges. 1894. XXVII. 497, 925.—Ch. C. 1894. I. 624, 864.— K. J. V. 114.
VI. *Ibid.* II.—*Ibid.* 3325.—Ch. C. 1895. I. 328.—K. J. VI. 50.
VII. Bemerkungen über den bei der Autodigestion der Hefe entstehenden Zucker.—Zeitschrift f. Biologie. 1895. XXXII. 468.—Ch. C. 1895. II. 870.— K. J. VI. 56.
VIII. Über das "Invertin" der Hefe.— Z. f. physiolog. Chemie. 1900. XXXI. 305.—Ch. C. 1901. I. 263.
IX. Über die Gärung der Pentosen.— Z. f. physiolog. Chemie. 1900. XXX. 478.—Ch. C. 1900. II. 920.
X. Maly's Jahresberichte. 1877. VII. 286.

Salomon, Alfred Gordon, und Mathew, W. de Vere,
I. Einfluss gewisser phosphorsaurer Salze auf die Alkoholgärung.—The Brewer's Journal. 1884. 149.—Z. g. Br. 1884. VII. 231.

Sanguineti, J.,
I. Contribution à l'étude de l'Amylomyces Rouxii de la levure chinoise et des moisissures ferments de l'amidon.—Ann. Inst. Past. 1897. XI. 264.—C. f. B. 2. Abt. 1897. III. 430.—Ch. C. 1897. I. 998.—K. J. VIII. 285.

Sanna-Salaris, G.,
See Malerba e Sanna-Salaris.

Sannino, Antonio,
See Sostegni e Sannino.

Sanson,
I. C. R. 1888. CVI. 208.

Sassen, A.,
See Woltering und Sassen.

Saussure, Théodore de,
I. Sur la fermentation vineuse.—Bibliothèque univ. de Genève. 1841. No. 63, p. 180.—Journal f. prakt. Chemie. 1841. XXIV. 47.

Sauvageau, C., et Radais, M.,
I. Sur les genres Cladothrix, Streptothrix, Actinomyces et description de deux streptothrix nouveaux.—Ann. Inst. Past. 1892. VI. 242.—C. R. 1892. CXIV. 559.—K. J. III. 45.

Savoff,
I. Recherches sur l'aspergillose pulmonaire. Nancy. 1905.

Savoure,
I. Arch. de Parasitologie. 1905.—Abst. in Bot. Centralbl. 1906. CI. 11.

Schacht, Hermann,
I. Die Kartoffelpflanze und deren Krankheiten. Berlin. C. Wigand.
II. Über die Veränderungen durch Pilze in abgestorbenen Pflanzenzellen.—Jahrbücher f. wissenschaftl. Botanik. 1863. III. 442.

Schaffer, F.,
I. Die Anwendung der eudiometrischen Methode zur Untersuchung von Milch, Lab und Wasser zu Käsereizwecken.—Schweizer. Wochenschrift f. Pharmacie. 1894. XXXII. 17.—Landw. Jahrbuch der Schweiz. 1893.—Ch. C. 1894. I. 607; II. 223.—Milchzeitung. 1894. XXIII. 349.
II. Über das Casein und die Wirkung des Labfermentes in der Kuhmilch.—Landw. Jahrb. d. Schweiz. 1887. I. 43.—Ch. C. 1887. 1359.
III. Über des Einfluss des sogen. Nachwärmens bei der Käsefabrikation auf die Reifungsprodukte der Käse.—Landw. Jahrbuch d. Schweiz. 1895. IX. 93.—C. f. B. 2. Abt. 1895. I. 760.
IV. Dissert. Erlangen. 1901, p. 15.
See also Freudenreich u. Schaffer; Hess, Schaffer u. Lang; Nencki und Schaffer.

Schaffer, F., und Bondzynski, St.,
I. Über das Casein und die Wirkung des Labfermentes in der Kuhmilch.—Landw. Jahrbuch d. Schweiz. 1887. I. 47. 1888. II. 32.—Ch. C. 1887. 1359.

Schaffer, F., und Freudenreich, Ed. von,
I. De la résistance des bactéries aux hautes pressions combinées avec une élévation de la température.—Annales de Micrographie. 1891. IV. 105.—K. J. II. 99.
II. Quantitative Untersuchungen über die in Naturweinen und Kunstweinen enthaltenen Hefen und Bakterien.—Landw. Jahrb. d. Schweiz. 1891. V. 79.—Ann. de Micrographie. 1892. V. 239.—C. f. B. 1892. XI. 467.—K. J. II. 62.

Schander, R.,
I. Jahresb. d. Vereinigung d. Vertreter d. angew. Botanik. 1903–4. 104.
II. Bericht d. Kgl. Lehranstalt zu Geisenheim f. d. Jahr. 1903–4, p. 123.
III. Jahresb. d. Vereinigung, etc., for 1903–4, 1905. 92.
IV. *Ibid.* for 1903–4, 1905. 85.

Schardinger, F.,
I. C. f. B. 2. Abt. 1890. VIII. 144.

Schardinger, Franz,
I. Reinkulturen von Protozoen auf festen Nährböden.—C. f. B. 2. Abt. 1896. XIX. 538.
II. Über eine neue, optisch aktive Modifikation der Milchsäure, durch bakterielle Spaltung des Rohrzuckers erhalten.—Monatshefte f. Chemie. 1890. XI 545.—Ch. C. 1891. I. 441.—K. J. I. 85.

Schedtler, H.,
I. Beitrag zur Morphologie der Bakterien. Bacterium Zopfii Kurth.—Virchow's Archiv f. patholog. Anatomie. 1887. CVIII. 30.—C. f. B. 1887. II. 437.

Scheele, Karl Wilhelm,
I. Anmärkningar om sättet att conservera Attika.—Kongl. Vetenskaps Academiens nya Handlingar. 1782. III. 120.
II. Crell's Annalen. 1785. I. 61.

Scheibler, C.,
I. Untersuchungen über die Natur der gallertartigen Ausscheidung (sog. "Froschlaich"), welche bei der Saftgewinnung aus Rübenbeobachtet wird.—Zeitschrift des Vereins f. d. Rübenzucker-Industrie des Deutschen Reiches. 1869. 472. 1874. 309. 1875. 112.—Biedermann's Centralblatt. 1875. VIII. 131.

II. Zur Geschichte der Melitriose.—Neue Zeitschrift für Rübenzucker-Industrie. 1889. XXIII. 234.—Ch. C. 1890. I. 21.

III. Über das Vorkommen der Arabinsäure (Gummi) in den Zuckerrüben und über den Arabinzucker (Gummizucker).—Ber. d. D. Chem. Ges. 1873. VI. 612.

SCHEIBLER, C., und MITTELMEIER, H.,

I. Über die Inversionsprodukte der Melitriose.—Ber. d. D. Chem. Ges. 1889. XXII. 1678, 3118.—Ch. C. 1889. II. 557. 1890. I. 110.

II. Ber. d. D. Chem. Ges. 1885. XVIII. 1779.

III. *Ibid.* 1889. XXII. 3120.

IV. Z. f. angew. Chemie. 1891. 407.

SCHELLHORN, B.,

See WINDISCH und SCHELLHORN.

SCHENCK,

I. W. f. Br. 1905. XXII. 221.

SCHENK, Samuel,

I. Über einen Micrococcus tetragenus concentricus in den Fäces.—Allgem. Wiener medic. Zeitschrift. 1892.

SCHERPE, R.,

I. Die chemischen Veränderungen des Roggens und Weizens beim Schimmeln und Auswachsen.—Zeitschrift f. Untersuchung d. Nahrungs- und Genussmittel. 1899. II. 550.—C. f. B. 2. Abt. 1900. VI. 747.—Ch. C. 1899. II. 398.

SCHEURER-KESTNER, A.,

I. Sur un ferment digestif qui se produit pendant la panification.—C. R. 1880. XC. 369.—Ch. C. 1880. 214.

SCHEURLEN,

I. Über Saprol und die Saprolierung der Desinfektionsmittel. — A. f. Hygiene. 1893. XVIII. 35.

II. Weitere Untersuchungen über Saprol. —A. f. Hygiene. 1893. XIX. 347.—Gives a copious bibliography for saprol. —Ch. C. 1892. II. 118, 580, 585, 727. 1893. I. 890; II. 869. 1894. I. 522.

III. Geschichtliche und experimentelle Studien über den Prodigiosus.—A. f. Hygiene. 1896. XXVI. 1. — Ch. C. 1896. I. 1013.—K. J. VII. 54.

SCHEURLEN und SPIRO,

I. Die gesetzmässigen Beziehungen zwischen Lösungszustand und Wirkungswert der Desinfektionsmittel.—Münchener medic. Wochenschrift. 1897. XXXXIV. 81. —Ch. C. 1897. I. 505.—K. J. VIII. 63.

SCHEWIAKOFF, W.,

I. Über einen neuen bakterienähnlichen Organismus des Süsswassers.—Verhandl. d. Naturhist.-med. Vereins Heidelberg. Bd. V.; auch Habilitationsschrift. Heidelberg. 1893. Winter.—C. f. B. 1893. XIV. 151.—K. J. IV. 57.

SCHIEL,

I. Elektrotherapeutische Studien. — Deutsches Archiv für klinische Medizin. 1875. XV. 190.

SCHIFFERER, Anton,

I. Über die nicht-kristallisierbaren Produkte der Einwirkung der Diastase auf Stärke. Dissert. Basel. 1892.—Ch. C. 1892. II. 1011.—K. J. III. 250.

SCHILD, E.,

I. Formalin zur Diagnose des Typhusbacillus.—C. f. B. 1893. XIV. 717.—Ch. C. 1894. I. 162.

II. Eine Typhus-Epidemie mit nachweisbarer Entstehungsursache und die Diagnose des Typhusbacillus mittelst Formalin.—Z. f. Hygiene. 1894. XVI. 368.—C. f. B. 1894. XV. 692.—Ch. C. 1894. I. 872.

SCHILOW, P.,

I. Einfluss des Wasserstoffsuperoxyds auf einige pathogene Mikroorganismen.—Pharmac. Zeitschrift f. Russland. 1894. XXXIII. 99.—Ch. C. 1894. I. 689.

SCHINDLER, S.,

I. Beiträge zur Kenntnis des Adenins, Guanins und ihrer Derivate.—Z. f. physiolog. Chemie. 1889. XIII. 432.—Z. g. Br. 1889. XII. 203.—Ch. C. 1889. I. 719.

SCHIÖNNING, H.,

I. Nouvelle et singulière formation d'ascus dans une levure.—Comptes rendus, etc., de Carlsberg. 1895. IV. 30.—C. f. B. 2. Abt. 1895. I. 441.—K. J. VI. 34.

II. Comptes rendus, etc., de Carlsberg. 1903. VI. 103.

III. Notice in Chem. Ztg. 1895. IX. 225.

See also KLÖCKER und SCHIÖNNING.

SCHIPIN, D.,

I. Zur Bakteriologie des Kumys. Dissert. St. Petersburg. 1899.—C. f. B. 2. Abt. 1900. VI. 775.

SCHIROKIKH, J.,

I. Über einen neuen Salpeter zerstörenden Bacillus.—C. f. B. 2. Abt. 1896. II. 204.—Ch. C. 1896. II. 111.—K. J. VII. 216.

SCHLEGEL,

I. Kupferkalkmischung und Traubenfäule. —Weinbau und Weinhandel. 1894. XII. 539.

SCHLEIDEN, Matthias Jakob,

I. Grundzüge der wissenschaftlichen Botanik. 3rd Ed. 1849. I. 207.

II. Beiträge zur Phytogenesis.—Müller's Archiv f. Anatomie, Physiologie u. wissenschaftl. Medicin. 1838. 137.

SCHLÖSING, Th.,

I. Sur la fermentation en masses du tabac pour poudre.—Mémorial des Manufactures de l'état. 1888. I. 514. 1889. II. 119.

II. Contribution à l'étude de la fermentation du râpé. Du rôle des transvasements. —*Ibid.* II. 192.

III. Sur la décomposition des nitrates pendant les fermentations.—C. R. 1868. LXVI. 237.—Ch. C. 1868. 940.

Schlösing, Th. fils, et Laurent, Emile,
I. Sur la fixation de l'azote libre par les plantes.—C. R. 1891. CXIII. 776, 1059. 1892. CXV. 659, 732.—Ann. Inst. Past. 1892. VI. 65, 824.—Ch. C. 1892. I. 170. 1893. I. 52.—K. J. II. 204, 206; III. 208.

Schlösing, Th., et Müntz, A.,
I. Sur la nitrification par les ferments organisés.—C. R. 1877. LXXXIV. 301; LXXXV. 1018.
II. Recherches sur la nitrification.—C. R. 1879. LXXXIX. 891, 1074.

Schlossberger, Julius Eugen,
I. Über die Natur der Hefe, mit Rücksicht auf die Gärungserscheinungen.—Annalen d. Chem. u. Pharm. 1844. LI. 193.

Schlossberger, J., und Doepping, O.,
I. Chemische Beiträge zur Kenntnis der Schwämme.—Annalen d. Chemie und Pharmacie. 1844. LII. 106.

Schmelck, L.,
I. Eine Gletscherbakterie.—C. f. B. 1888. IV. 545.—Ch. C. 1889. I. 48.

Schmid, E.,
See Nobbe, Hiltner und Schmid; Nobbe, Schmid, Hiltner u. Hotter.

Schmidt, B.,
I. Über den Einfluss der Bewegung auf das Wachstum und die Virulenz der Mikroben. —A. f. Hygiene. 1891. XIII. 247.—K. J. II. 73.

Schmidt, C.,
I. Liebig's Ann. 1847. LXI. 171.
II. Handwörterbuch der Chemie. 1848. III. 224.

Schmidt, Paul,
I. Milch, die Quelle einer Typhusepidemie. Dissert. Halle. 1893.—C. f. B. 1894. XV. 63.

Schmidt, R.,
I. Über verschimmelte Tapeten. Dissert. Erlangen. 1899.—C. f. B. 1. Abt. 1900. XXVII. 752.

Schmidt, R. H.,
I. Über Aufnahme und Verarbeitung von fetten Ölen durch Pflanzen.—Flora. 1891. LXXIV. 300.—Ch. C. 1892. I. 320.
II. Sitzgsber. d. physik. med. Societät Erlangen. 1898. Heft 30.

Schmidt Mühlheim,
I. Untersuchungen über fadenziehende Milch.—Pflüger's Archiv. 1882. XXVII. 490. — Die landw. Versuchs-Stationen. 1892. XXVIII. 91.—Ch. C. 1882. 566. —Z. f. Spiritusind. 1882. V. 312.

Schmidt-Nielsen,
I. C. f. B. 2. Abt. 1902. IX. 145.

Schmiedeberg, O., und Harnack, E.,
I. Synthesen des Muscarins und über muscarinartig wirkende Ammoniumbasen. —Ch. C. 1876. 554.
See also Bergmann und Schmiedeberg.

Schmitz, Friedrich,
I. Über die Zellkerne der Thallophyten.—Verhandl. u. Sitzungsber. d. natur-hist. Vereins d. preuss. Rheinlande und Westphalens. 1879. XXXVI. 345.
II. Untersuchungen über die Struktur des Protoplasmas und der Zellkerne der Pflanzenzellen.—*Ibid.* 1880. XXXVII. 159.

Schmitz, J.,
I. Beiträge zur Anatomie und Physiologie der Schwämme.—Linnæa. 1843. XVII. 417.

Schmitz-Dumont, W.,
See Schroeder u. Schmitz-Dumont.

Schmoeger, M.,
I. Über Centrifugen-Käse.—Milchzeitung. 1883. XII. 483.

Schneider, A.,
I. Observations on some American Rhizobia.—Bull. of the Torrey Botan. Club. 1892. July.—K. J. III. 207.—Compare Ber. d. D. Bot. Ges. 1894. XII. 11.—Zeitschrift f. Pflanzenkrankheiten. 1895. V. 232.

Schneider, Paul,
I. Die Bedeutung der Bakterienfarbstoffe für die Unterscheidung der Arten. Inaugural-Dissertation. Basel. 1894.—C. f. B. 2. Abt. 1895. I. 633.—K. J. V. 62.

Schnell,
I. Erfahrungen bei der Hefereinzucht unter Verwendung rein gezüchteter Hefen zur Weinvergärung. — Zeitschrift f. angewandte Chemie. 1894. 417.—Ch. C. 1894. II. 498.—K. J. V. 173.

Schnirer, M. T.,
I. Zur Frage nach der Verbreitung der Tuberkelbacillen ausserhalb des Körpers. —Wien. medic. Presse. 1891. 3.—C. f. B. 1891. IX. 544.

Schnurmans Stekhoven, J. H.,
I. Saccharomyces Kefyr. Proefschrift. Utrecht. 1891.—K. J. II. 136.

Schönbein, Christian Friedrich,
I. Über Ozon und Ozonwirkungen in Pilzen. —Phil. Magazine. XI. No. 70. 137.—Journal f. prakt. Chemie. 1856. LXVII. 496.

Schönfeld, Franz,
I. Das Hefenwachstum in der Hauptgärung bei untergärigem Bier.—W. f. Br. 1896. XIII. 421.—C. f. B. 2. Abt. 1896. II. 462.—Ch. C. 1896. I. 1181.—K. J. VII. 89.
II. Über die Gärungs- und Nachgärungs-Verhältnisse mit besonderer Berücksichtigung des tatsächlichen Auftretens von Infektion in den obergärigen Brauereien Nord- und Mitteldeutschlands.—W. f. Br. 1895. XII. 546.—C. f. B. 2. Abt. 1895. I. 639.—K. J. VI. 208.
III. Das Infizieren von Fiaschenbier durch Sarcina.—W. f. Br. 1897. XIV. 177.—C. f. B. 2. Abt. 1899. V. 162.—K. J. VIII. 142.
IV. Studien über eine Bier-Sarcina.—W. f. Br. 1898. XV. 285.—K. J. IX. 136.

V. Erforschung der Quellen der Sarcina-Infektion im Brauereibetrieb.—W. f. Br. 1898. XV. 321.—C. f. B. 2. Abt. 1898. IV. 865.—K. J. IX. 138.

VI. Welche praktischen Massnahmen sind zu ergreifen zur Bekämpfung der Sarcina-Infektion.—W. f. Br. 1898. XV. 694. —K. J. IX. 140.

VII. Studien über eine Biersarcina.—W. f. Br. 1899. XVI. 665.—C. f. B. 2. Abt. 1900. VI. 262.—K. J. X. 154.

VIII. Einige Versuche zur Fortzüchtung verschiedener Sarcinen Rassen.—W. f. Br. 1899. XVI. 681.—C. f. B. 2. Abt. 1900. VI. 376.—K. J. X. 157.

IX. Die Bakterieninfektionen bei den obergärigen Bieren.—W. f. Br. 1901. XVIII. 274.—C. f. B. 2. Abt. 1902. VIII. 282.

X. Die Stellhefe des Berliner Weissbieres.—W. f. Br. 1902. XIX. 146.—C. f. B. 2. Abt. 1902. IX. 168.

XI. W. f. Br. 1898. XV. 288.

Scholl, Hermann,

I. Untersuchungen über giftige Eiweisskörper bei Cholera asiatica und einigen Fäulnisprozessen.—A. f. Hygiene. 1892. XV. 172.—C. f. B. 1892. XII. 727.—Ch. C. 1892. II. 795.

II. Die Milch. Wiesbaden. 1891.

Scholtz, W.,

I. Über den Nachweis von Arsen auf biologischen Wege in den Hautschuppen, Haaren, Schweiss und Urin.—Berliner klin. Wochenschrift. 1899. XXXVI. 913.—Ch. C. 1899. II. 1033.

Schostakowitsch, W.,

I. Über die Bedingungen der Konidien-Bildung bei Russthau-Pilzen. — Flora. 1895. LXXXI. 362.—C. f. B. 2. Abt. 1896. II. 235.

Schottelius, M.,

I. Biologische Untersuchungen über den Micrococcus prodigiosus. Leipzig. 1887. Engelmann.—C. f. B. 1887. II. 439.

Schrank, J.,

I. Untersuchungen über den im Hühnerei die stinkende Fäulnis hervorrufenden Bacillus. — Wiener medic. Jahrbücher. 1888. 303.—C. f. B. 1889. V. 770.—Ch. C. 1889. II. 463.

Schreiner,

I. Über Kuhmilch, etc.—Tageblatt d. 50 Vers. D. Naturforscher u. Ärzte. 1877. —Ch. C. 1878. 588.

Schrohe, A.,

I. Behandlung von Wasser und alkoholischen Getränken mit Elektrizität, Ozon und Wasserstoffsuperoxyd zur Reinigung, Konservierung und Geschmacksverbesserung.—W. f. Br. 1891. VIII. 706.

II. Über einen 18 Proz. Alkohol ergebenden Gärungserreger.—Z. f. Spiritusind. 1891. XIV. 96.—C. f. B. 1. Abt. 1892. XII. 467.

Schröder, H.,

I. Über Filtration der Luft in Beziehung auf Fäulnis, Gärung und Krystallisation.—Annalen der Chemie u. Pharmacie. 1859. CIX. 35. 1861. CXVII. 273.—Ch. C. 1859. 410. 1861. 542.

Schröder, H., und Dusch, Th. von,

I. Über Filtration der Luft in Bezug auf Fäulnis und Gärung.—Annalen der Chemie u. Pharmacie. 1854. LXXXIX. 232.—Ch. C. 1854. 303.

Schroeder, J. von,

I. Über Gerbung mit Fichten extrakt und Quebracho-Extrakt.—Deutsche Gerberzeitung. 1889. No. 38.

Schroeder, J. von, und Bartel, A.,

I. Über Gerbstoffverluste beim Gären der Gerbebrühen.—Deutsche Gerberzeitung. 1890. No. 67.

Schroeder, J. von, und Schmitz-Dumont, W.,

I. Beiträge zur Kenntnis der chemischen Natur der "Ascher."—Dingler's Journal. 1896. CCC. 161.—Ch. C. 1896. II. 278.

Schroeter, J.,

I. Über einige durch Bakterien gebildete Pigmente.—Cohn's Beiträge z. Biologie d. Pflanzen. 1870. Bd. I. Heft 2. 109.

Schröter,

I. In Rabenhorst's Kryptogamenflora Schlesiens. III. 220.

II. *Ibid.* III. 218.

Schröter u. Cohn,

I. Beiträge z. Biol. d. Pflanz. 1872. I. 110, 187. Pl. III. fig. 1.

Schrötter, Hermann von,

I. Vorläufige Mitteilungen über das Pigment von Sarcina aurantiaca und Staphylococcus pyogenes aureus.—C. f. B. I. Abt. 1895. XVIII. 781.—Ch. C. 1896. I. 317.

Schrötter, Hugo,

I. Über eine im Fuselöl enthaltene Base.—Ber. d. D. Chem. Ges. 1879. XII. 1431.

Schüpphaus,

I. Journ. Am. Chem. Soc. 1892. XIV. 45.

Schütte,

See Tacke, Immendorf, Hessenland, Schütte und Minssen.

Schütz, J.,

I. Hofmeister's Beiträge z. chem. Physiologie u. Pathologie. 1903. III. 433.

Schützenberger, Paul,

I. Recherches sur la levure de bière. — Bulletin de la Société chimique de Paris. 1874. XXI. 204.—C. R. '874. LXXVIII. 493, 698.

II. Die Gärungserscheinungen. 1876.

III. Ber. d. D. Chem. Ges. 1874. VII. 192.

Schützenberger, P., et Destrem, A.,

I. Recherches sur la levure de bière.—C. R. 1879. LXXXVIII. 287.—Z. g. Br. 1879. II. 271.

Schützenberger, P., et Quinquaud, E.,

I. Sur la respiration des végétaux aquatiques immergés.—C. R. 1873. LXXVII. 272.

Schukow, J.,
I. Über reine Weinhefen.—W. f. Br. 1899. XVI. 195.—C. f. B. 2. Abt. 1899. V. 411.
II. C. f. B. 2. Abt. 1896. II. 611.

Schlute im Hofe,
I. Über den Einfluss der Milchsäure bezw. Schwefelsäure auf den Stickstoffgehalt der Maische.—Z. g. Br. 1889. XII. 325.—Ch. C. 1889. II. 955.

Schultz,
I. Über das Umschlagen der Rotweine.—Weinlaube. 1877. IX. 303.

Schultz, N. K.,
I. Zur Frage von der Bereitung einiger Nährsubstrate.—C. f. B. 1891. X. 52.—K. J. II. 26.

Schultze, W.,
I. Über die Zerstörung des Biergeschmackes und- Geruches durch das Sonnen- oder Tageslicht im Kleinverkehr mit Bier.—Mitteilungen d. österr. Versuchs-Station f. Brauerei und Mälzerei in Wien. 1888. I. 10.—Z. g. Br. 1890. XIII. 162.
II. Über den Vergärungsgrad der Hauptgärung.—Der. bayr. Bierbrauer. 1876. 22.

Schulz, A.,
I. Über den Stoffbedarf und den Stoffumsatz des Kahmpilzes (Saccharomyces Mycoderma). — Annalen der Önologie. 1878. VII. 115.—Just's Botan. Jahresbericht. 1879. 84.

Schulz, Hugo,
I. Über Hefegifte.—Pflüger's Archiv. 1888. XXXXII. 517.—Ch. C. 1888. 583.—Z. g. Br. 1888. XI. 183.
II. Zur Kenntnis der Oxydation der Fette.—Pflüger's Archiv. 1877. XXXX. 398.—Ch. C. 1878. 47.

Schulze, Carl,
I. Die Anwendung des Pasteurisierens gegen Nachgärungen der Weine auf den Flaschen.—Landw. Jahrbücher. 1895. XXIV. 403.—C. f. B. 2. Abt. 1895. I. 833.—K. J. V. 202.

Schulze, Ernst,
I. Über die Cellulose.—Chemiker-Zeitung. 1895. XIX. 1465.—Ch. C. 1895. II. 595.
II. Die chemische Zusammensetzung der pflanzlichen Zellmembranen.—Ber. d. D. Chem. Ges. 1891. XXIV. 2277.—Ch. C. 1891. II. 472.
III. Ber. d. D. Chem. Ges. 1890. XXIII. 2579.
IV. Chem. Ztg. 1893. XVII. 1263.
V. Ber. d. D. Chem. Ges. 1889. XXII. 1191. 1891. XXIV. 2277.
See also Benecke und Schulze, Röse und Schulze.

Schulze, Franz,
I. Vorläufige Mitteilung der Resultate einer experimentellen Beobachtung über generatio æquivoca.—Poggendorff's Annalen d. Physik u. Chemie. 1836. XXXIX. 487.

Schulze, Franz Ferdinand,
I. Beiträge zur Kenntnis des Lignins. Rostock. 1856.

Schumacher, Emil,
I. Beiträge zur Morphologie und Biologie der Hefe.—Sitzungsberichte d. K. Akademie der Wiss. in Wien. Mathem.-naturw. Klasse. 1874. LXX. I. 157.

Schunck, Edward,
I. Über die Einwirkung des Krapp-Ferments auf Zucker.—Journal f. prakt. Chemie. 1854. LXIII. 222.—Ber. d. D. Chem. Ges. 1898. XXXI. 309.—Ch. C. 1898. I. 899.—K. J. IX. 319.
II. Mem. Manchester Lit. and Phil. Soc. 1853–54.—Ber. d. D. Chem. Ges. 1898. XXXI. 309.

Schuppan, P.,
I. Die Bakteriologie in ihrer Beziehung zur Milchwirtschaft.—C. f. B. 1893. XIII. 527.—Ch. C. 1893. I. 1033.—K. J. IV. 179.

Schuurmans-Stekhoven, J. H.,
I. Dissert. Utrecht. 1891.—Onderzoekingen gedonn in het Physiolcg. Laborat. des Utrechtschen Hoogschol. Edited by T. W. Engelmann and C. A. Pekelharing. 4th Series. I. 2, p. 119.

Schwanhaeuser, Rudolf,
I. Beitrag zur experimentellen Untersuchung der Ursache der Gesundheitsschädlichkeit hefetrüber Biere. — Dissert. Greifswald. 1890.—C. f. B. 1. Abt. 1891. IX. 100.

Schwann, Theodor,
I. Vorläufige Mitteilung betreffend Versuche über Weingärung und Fäulnis.—Poggendorff's Annalen d. Physik u. Chemie. 1837. XXXXI. 184.
II. Mikroskopische Untersuchungen über die Übereinstimmung in der Textur und dem Wachstum der Tiere und Pflanzen. Berlin. 1839.
III. Über das Wesen des Verdauungsprozesses. — Joh. Müller's Archiv für Anatomie, Physiologie, etc. 1836. 90.—Poggendorff's Annalen. 1836. XXXVIII. 358.

Schwarz,
I. Liebig's Ann. 1852. LXXXIV. 82.

Schweinitz, E. A. von,
I. Kulturmedien für biochemische Untersuchungen. — The New York Medical Journal. 1893.—C. f. B. 1893. XIV. 330.

Schweitzer, R.,
See Bleisch und Schweitzer.

Schweizer, Eduard,
I. Das Kupferoxyd-Ammoniak, ein Auflösungsmittel für die Pflanzenfaser.—Vierteljahrsschrift d. Züricher Naturf. Ges. 1857. II. 395. — Journal f. prakt. Chemie. 1857. LXXII. 109.

Schweninger, F.,
I. Über die Wirkung faulender org. Substanzen auf den lebenden tierischen Organismus. — Gekrönte Preisschrift. München. 1866.

Schwickerath, Karl,
I. German Patent No. 113164, April 8, 1899.—Ch. C. 1900. II. 653.

Sclavo, Achille, und Gosio, B.,
I. Über eine neue Gärung des Stärke.—Staz. sperim. agrar. ital. 1890. XIX. 540.—Ch. C. 1891. II. 253.—K. J. II. 242.

Sédillot, Charles Emmanuel,
I. De l'influence des découvertes de M. Pasteur sur les progrès de la chirurgie.—C. R. 1878. LXXXVI. 634.

Seekamp, W.,
I. Über die Zersetzung der Weinsäure und Citronensäure durch das Sonnenlicht.—Annalen der Chemie und Pharmacie. 1894. CCXLVIII. 373.—Ch. C. 1894. I. 854.

Segall,
See Buchner und Segall.

Seifert, Wenzel,
I. Die Organismen der alkoholischen Gärung in der Weinbereitung.—Weinlaube. 1901. XXXIII. 2.
II. Ber. d. chem. phys. Versuchsst. Klosterneuberg. 1899–1900.—Zeitschr. f. d. Landw. Versuchsw. in Österreich. 1900. Heft 3.
IV. Z. f. Nährungsmittel-Unters., etc. VII. 148.
V. Zeitschr. f. d. landw. Versuchswesen in Österreich. 1901. IV. 221.
VI. *Ibid.* 1906. IX. 1019.

Seiter, O.,
I. Studien über die Abstammung der Saccharomyceten und Untersuchungen über Schizosaccharomyces octosporus. Dissert. Erlangen. 1896.—C. f. B. 2. Abt. 1896. II. 301.—Ch. C. 1896. II. 250.—K. J. VII. 32.

Sell, Eugen,
I. Über Cognac, Rum und Arak. I. Mitteilung (Cognac).—Arbeiten a. d. Kaiserl. Gesundheitsamte zu Berlin. 1890. VI. 335.—Ch. C. 1890. II. 637.
II. *Ibid.* II. Mitteilg. (Rum u. Arak).—*Ibid.* 1891. VII. 210.—Ch. C. 1891. I. 1097.

Selmi, Francesco,
I. Preparazione nuova dell' acido lactico, etc. Milano. 1844.—Journal de Pharmacie et de Chimie. 1846. X. 458.
II. Sulle ptomaïne ed alkaloidi cadaveri. Bologna. 1878.—Ber. d. D. Chem. Ges. 1878. XI. 808, 1691.
III. Nuovo processo generale per la ricerca delle sostanze venefiche. Bologna. 1875.—Ber. d. D. Chem. Ges. 1874. VII. 1642.

Senus, A. H. C. van,
I. Bijdrage tot de kennis der cellulosegisting.—Proefschrift. Leiden. 1890.—K. J. I. 136.

Serafini, A.,
I. Chemisch-bakteriologische Analysen einiger Wurstwaren. Ein Beitrag zum Studium der Nahrungsmittel-Konservierung.—A. f. Hygiene. 1891. XIII. 173.—K. J. II. 106.

Serafini, A., und Ungaro, G.,
I. Der Einfluss des Räucherns auf die Lebensfähigkeit der Bakterien.—Ann. dell' Ist. d'Igiene di Roma. 1891. II.—K. J. II. 106.

Sestini, Fausto e Leone,
I. Über die ammoniakalische Gärung der Harnsäure.—D. Landw. Versuchs-Stationen. 1890. XXXVIII. 157.—Ch. C. 1890. I. 1064.—K. J. I. 100.

Sette,
I. Memoria storico-naturale sull' arrossimento straordinario di alcune sostanze alimentose, osservato nella provincia di Padova l'anno 1819 letta all' Ateneo di Treviso, nella sera 28 Aprile 1820. Venezia. 1824.—Schweigger's Journal f. Chemie u. Physik. 1827. L. 396.

Severin, S. A.,
I. Die im Miste vorkommenden Bakterien und deren physiologische Rolle bei der Zersetzung desselben.—C. f. B. 2. Abt. 1895. I. 97, 799.—Ch. C. 1896. I. 378.

Seyffert, H.,
I. Einiges über Reinzucht-Hefen und ihre Ernährung.—Z. g. Br. 1896. XIX. 318.—C. f. B. 2. Abt. 1896. II. 465.—K. J. VII. 123.
II. W. f. Br. 1904. XXI. 519.

Seynes, J. de,
I. Sur le Mycoderma vini.—C. R. 1868. LXVII. 105.—Bot. Ztg. 1869. XXVII. 521.

Shibata,
I. Beiträge z. Chem. Physiologie u. Pathologie. 1904. V. 384.

Shiga, K.,
I. Z. f. physiolog. Chemie. 1904. XLII. 502.

Siebel,
I. Nutzbarmachung der Hefe.—Ch. C. 1898. I. 1049.—K. J. IX. 104.

Siebenmann,
I. Die Fadenpilze Aspergillus u. Eurotium. 1882. 2nd Ed. as Die Schimmelmykosen des menschlichen Ohres. Wiesbaden. 1889.

Sieber, Nadina,
I. Über die antiseptische Wirkung der Säuren.—Journal f. prakt Chemie. 1879. XIX. 433.—Ch. C. 1879. 525.
II. Die angebliche Umwandlung von Eiweiss in Fett beim Reifen des Käses.—Journal f. prakt. Chemie. (2.) 1880. XXI. 203.
See also Macfadyen, Nencki u. Sieber; Nencki und Sieber.

Sigmund, Wilhelm,
I. Über fettspaltende Fermente im Pflanzenreiche.—Monatshefte f. Chemie. 1890. XI. 272.—Ch. C. 1890. II. 628.

II. Beziehungen zwischen fettspaltenden und glykosidspaltenden Fermenten.—*Ibid.* 1892. XIII. 567.—Ch. C. 1892. II. 579.

Simácek, E.,
I. Centralbl. f. Physiologie. 1903. XVII. 477.
II. *Ibid.* XVII. 209.

Simanowsky, N. P.,
I. Über die Gesundheitsschädlichkeit hefetrüber Biere und über den Ablauf der künstlichen Verdauung bei Bierzusatz.—A. f. Hygiene. 1886. IV. 1.—Reproduced in Z. g. Br. 1886. IX. 115.

Sirotinin,
See Flügge und Sirotinin.

Sitnikoff, A., und Rommel, W.,
I. Vergleichende Untersuchungen über einige sogen. Amylomyces-Arten.—Z. f. Spiritusind. 1900. XXIII. 391.—W. f. Br. 1900. XVII. 621.—C. f. B. 2. Abt. 1901. VII. 245.

Sjöbring, Niels,
I. Über Kerne und Teilungen bei den Bakterien.—C. f. B. 1892. XI. 65.—K. J. III. 35.

Skalweit,
I. Bier-Untersuchungen.—Z. g. Br. 1878. I. 476. 1879. II. 331. 1880. III. 68.

Skerst, Oskar von,
I. Beiträge zur Kenntnis des Dematium pullulans.—W. f. Br. 1898. XV. 354.—C. f. B. 2. Abt. 1898. IV. 864.

Slater, C.,
I. On a Red-Pigment-forming Organism, Bacillus corallinus. — The Quarterly Journal of Microscopical Science. 1891. Vol. 32. Part III.—K. J. II. 43.

Smith, Allen J.,
I. A New Chromogenic Bacillus, Bacillus cœruleus. — Medical News. 1887. II. 758.—C. f. B. 1888. III. 401.

Smith, Claud,
See Cross, Bevan and Smith.

Smith, Theobald,
I. Das Gärungskölbchen in der Bakteriologie.—C. f. B. 1890. VII. 502.—K. J. I. 14.
II. The Fermentation Tube, with Special Reference to Anaërobiosis and Gas Production among Bacteria.—Reprint from the Wilder Quarter-Century Book. Ithaca. 1893, p. 187.

Soldan, Carl,
I. Die Gesamtarbeitsleistung der Hefen Saaz, Frohberg und Logos in Saccharose-, Dextrose- und Maltose-Lösung unter verschiedenen Versuchs- und Ernährungsbedingungen. Dissert. Erlangen. 1897.—K. J. IX. 82.

Söldner, Friedrich,
I. Die Salze der Milch und ihre Beziehungen zu dem Verhalten des Caseins.—D. Landw. Versuchs-Stationen. 1888. XXXV. 351.

Sommaruga, G. von,
I. Über Stoffwechselprodukte von Mikroorganismen. III. — Deutsche med. Wochenschrift. 1894. No. 48.—Ch. C. 1895. I. 96.—K. J. V. 74.

Sonntag, Hermann,
I. Über die Bedeutung des Ozons als Desinficienz.—Z. f. Hygiene. 1890. VIII. 95.—C. f. B. 1890. VIII. 778.

Sorauer, Paul,
I. Handbuch der Pflanzenkrankheiten. 2nd Ed. Berlin. 1886.—I. Bd.: Die nichtparasitären Krankheiten. 20*s.*, bound. — II. Bd.: Die parasitären Krankheiten. 14*s.*, bound.
II. Die bakteriose Gummosis der Zuckerrüben. — Zeitschrift f. Pflanzenkrankheiten. 1892. 280.—Blätter für Zuckerrübenbau. 1894. p. 9.—C. f. B. 2. Abt. 1895. I. 295.
III. Handbuch der Pflanzenkrankheiten. 3rd Ed. 1906. Bd. II.

Sorel, E.,
I. Etude sur l'Aspergillus oryzæ.—C. R. 1895. CXXI. 948.—C. f. B. 2. Abt. 1896. II. 120.—K. J. VI. 41.

Sorokin, N.,
I. Eine neue Spirillum-Art.—C. f. B. 1887. I. 466.
II. Noch einmal über Spirillum endoparagogicum.—C. f. B. 1890. VII. 123.—K. J. I. 19.
III. Zur Frage über das Ferment von Kumys.—Tagebuch d. Gesellschaft d. Ärzte der Universität Kasan. 1883.—Just's Botan. Jahresbericht pro 1885. I. 193.

Soskin, Selik,
I. Kritische Geschichte der Lehre von der Fettbildung.—Journal f. Landwirtschaft. 1894. XLII. 157.

Sostegni, Livio, e Sannino, Antonio,
I. Über die Entstehung von Schwefelwasserstoff bei der Alkohol-Gärung.—Staz. sper. agr. ital. 1890. XVIII. 434.—Ch. C. 1890. II. 112.—K. J. I. 66.
See also Berlese e Sostegni.

Soxhlet, Franz,
I. Ein verbessertes Verfahren der Milchsterilisierung. — Münchner medic. Wochenschrift. 1891. No. 19 u. 20.—C. f. B. 1891. X. 203.—K. J. II. 187.
II. Chem. Ztg. 1899. XXIII. 851.

Soyka, J.,
I. Über ein Verfahren, Dauerpräparate von Reinkulturen auf festem Nährboden herzustellen.—C. f. B. 1887. I. 542.

Soyka, J., und Král, F.,
I. Vorschläge und Anleitungen zur Anlegung von bakteriologischen Museen.—Z. f. Hygiene. 1888. IV. 143.—C. f. B. 1888. IV. 188.

SPAETH, Eduard,
I. Nachweis des Mutterkorns im Mehl.—Pharmac. Central-Halle. 1896. XVII. 542.—Chem.-Zeitg. Repertorium. 1896. XX. 244.
II. Die chemische und mikroskopische Untersuchung des Harnes. Leipzig. 1897.

SPAETH,
See PROCHOWNIK u. SPAETH.

SPALLANZANI, Lazaro,
I. Dissertazione di fisica animale e vegetabile. Modena. 1765.
II. Opuscoli di fisica animale e vegetabile. Modena. 1776. German translation by Donndorf. Leipzig. 1779.

SPIECKERMANN u. BREMER,
I. Landw. Jahrbücher. 1901. XXXI. 81.

SPILKER, W., und GOTTSTEIN, A.,
I. Über die Vernichtung von Mikroorganismen durch die Induktionselektrizität.—C. f. B. 1891. IX. 77.—Ch. C. 1891. I. 549.—K. J. II. 96.

SPINA, A.,
I. Bakteriologische Versuche mit gefärbten Nährsubstanzen.—C. f. B. 1887. II. 71.

SPIRO,
See SCHEURLEN und SPIRO.

SPITTA, Albert,
See BUCHNER und SPITTA.

SPRENGEL, Karl,
I. Die Lehre vom Dünger. Leipzig. 1839.

STÄDELER, G.,
I. Untersuchungen über das Fibroïn, Spongin und Chitin, nebst Bemerkungen über den tierischen Schleim.—Annalen der Chemie und Pharmacie. 1859. CXI. 12.

STAGNITTA-BALISTRERI,
I. Die Verbreitung der Schwefelwasserstoffbildung unter den Bakterien.—A. f. Hygiene. 1893. XVI. 10.—C. f. B. 1893. XIII. 755.—K. J. IV. 99.

STAHL, Georg Ernst,
I. Zymnotechnia fundamentalis sive fermentationis theoria generalis. Halle. 1697. — German translation: Frankfurt. 1734.

STAHL, J.,
I. Formalin. — Pharmaceutische Zeitg. 1893. XXXVIII. 173.—Ch. C. 1893. I. 750.

STANLEY, Arthur,
See FRANKLAND, STANLEY and FREW.

STAVENHAGEN, A.,
I. Zur Kenntnis der Gärungserscheinungen.—Ber. d. D. Chem. Ges. 1897. XXX. 2422, 2963.—Ch. C. 1897. II. 1188. 1898. I. 395.—K. J. VIII. 281; IX. 314.

STEENBUCH, Chr.,
I. Zur mikroskopischen Untersuchung des Mehles. Eine Methode, wodurch die Gewebselemente leicht isoliert werden können.—Ber. d. D. Chem. Ges. 1881. XIV. 2449.—Ch. C. 1882. 60.

STEICKEL, C.,
I. Engl. Patent No. 5124 of 1895.

STEINHAUS, J. Th.,
I. Beitrag zur Lehre von den sogenannten sporogenen Körnern. — Sitzungsprotokolle der biolog. Sektion der Warschauer Naturforscher-Gesellsch. 1889.—Biolog. Centralblatt. 1889. IX. 574.

STEINHOF,
I. Über das Blauwerden der Milch.—Neue Annalen der Mecklenburg. Landwirtschaftsgesellschaft. Rostock. 1838. XXII. 512.

STEINMETZ, C.,
I. Kurze Mitteilungen über einige Versuche zur Frage der fäulniswidrigen Eigenschaften der Kohlensäure. — C. f. B. 1894. XV. 677.—Ch. C. 1894. II. 252. —K. J. V. 97.

STERLING, S.,
I. Die peptonisierenden Bakterien der Kuhmilch.—C. f. B. 2. Abt. 1895. I. 473.—Ch. C. 1895. II. 608.

STERN, Arthur L.,
I. The Nutrition of Yeast.—Transactions of the Chemical Society. 1899, p. 201. —Ch. C. 1899. I. 132.—K. J. IX. 84.
II. The Nutrition of Yeast. Part II.: On the Odour of the Gases evolved during Fermentation.—Journal of the Federated Institutes of Brewing. 1899. V. 399.—K. J. X. 109.
III. The Nutrition of Yeast. Part III.—Transactions of the Chemical Society. 1901. LXXIX. 943.—Ch. C. 1901. II. 139.
IV. Some Final Considerations on the Nutrition of Yeast. Part IV.—Journal of the Federated Institutes of Brewing. 1902. VIII. 690.
V. J. Federated Institutes of Brewing. 1902. No. 16. 199.—W. f. Br. 1903. XX. 198.
VI. Proc. Chem. Soc. 1898. CXCVIII. 182.

STETTNER, Th.,
I. Das Antinonnin. Ein neues Mittel gegen den Hausschwamm und andere Pilze.—Süddeutsche Bauzeitung. 1892. No. 60.—Z. g. Br. 1893. XVI. 6.—Ch. C. 1893. I. 395.—K. J. IV. 114.

STEUBER, L.,
I. Über die desinfizierende Wirkung von gelöschtem Kalk auf Hefe.—Z. g. Br. 1896. XIX. 41.—C. f. B. 2. Abt. 1896. II. 163.—Ch. C. 1896. I. 582.—K. J. VII. 132.
II. Beiträge zur Kenntnis der Gruppe Saccharomyces anomalus.—Z. g. Br. 1900. XXIII. 3.—C. f. B. 2. Abt. 1900. VI. 217.

Steudel, H.,
I. Über die Konstitution des Thymins.—Z. f. physiolog. Chemie. 1900. XXX. 539.—Ch. C. 1900. II. 1152. 1901. I. 443.

Stevens, F. L.,
I. The Effect of Aqueous Solutions upon the Germination of Fungus Spores.—Botanical Gazette. 1898. XXVI. 377.—C. f. B. 2. Abt. 1899. V. 610.

Stift, A.,
I. Über die pflanzlichen Schädlinge der Zuckerrübe.—C. f. B. 2. Abt. 1895. I. 489.
II. Über tierische Schädlinge der Zuckerrübe.—C. f. B. 2. Abt. 1895. I. 398.

Stocký, Alb.,
See Hanus und Stocký.

Stoecklin, Henri de,
I. Recherches cliniques et expérimentales sur le rôle des levures trouvées dans les angines suspectes de diphtérie.—Archives de médecine expérimentale et d'anatomie pathologique. 1898, p. 1.—Baumgarten's Jahresbericht. 1897. XIII. 748.

Stoehr, C.,
See Brandes und Stoehr.

Stöhr,
I. J. f. pr. Chem. 1893. XLVII. 439.

Stoklasa, Julius,
I. Chemische Untersuchungen auf dem Gebiete der Phytopathologie.—Z. f. physiolog. Chemie. 1895. XXI. 79.—C. f. B. 2. Abt. 1896. II. 126.
II. Studien über die Assimilation elementaren Stickstoffes durch die Pflanzen.—Landw. Jahrb. 1895. XXIV. 827.—Ch. C. 1896. I. 253.
III. Österr. Chem.-Ztg. 1903. VI. 289.
IV. Ber. d. D. Bot. Ges. 1904. XXII. 460.
V. *Ibid.* 1905. XXXVIII. 664.
See also Vanha und Stoklasa.

Stoklasa, J., u. Czerny,
I. Ber. d. D. Chem. Ges. 1903. XXXVI. 622.
II. *Ibid.* XXXVI. 4058.

Stoklasa, J., Cerny, F., Jelinek, J., u. Vitek,
I. C. f. B. 2. Abt. 1904. XIII. 86.
II. Zeits. f. d. landw. Versuchswesen in Österr. 1904. VII. 755.

Stoklasa, J., Jelinek, und Vitek,
I. Beiträge z. chem. Physiologie u. Pathologie. 1903. III. 460.

Stoklasa, J., u. Simáček, E.,
I. Centralbl. f. Physiologie. 1903. XVII. 209.

Stoll, O.,
I. Beiträge z. morphol. u. biolog. Characteristik v. Penicillium arten. Dissert. Würzburg. 1904.

Stone, W. E.,
I. Zur Kenntnis der Pentaglucosen.—Ber. d. D. Chem. Ges. 1890. XXIII. 3791.—Ch. C. 1891. I. 313.

Stone, W. E., und Tollens, B.,
I. Gärungsversuche mit Galactose, Arabinose, Sorbose und anderen Zuckerarten.—Annalen der Chemie. 1888. CCXXXXIX. 257.—Ch. C. 1889. I. 316.

Storch, W.,
I. Untersuchungen über Butterfehler und Säuerung des Rahmes.—Milchzeitung. 1890. XIX. 304.—K. J. I. 85.
See also Friis, Lunde und Storch.

Straub,
I. Forschungsber. u. Lebensmittel. 1895. II. 382.

Strecker,
I. Liebig's Ann. 1854. XCII. 80.

Strohmer, F.,
I. Bakterienwirkungen in der Zuckerfabrikation.—Österr.-ungar. Zeitschr. f. Zuckerindustrie und Landwirtschaft. 1891. XX. 7.—Neue Zeitschr. f. Rübenzuckerindustrie. 1891. XXVI. 161.—Ch. C. 1891. I. 897.—K. J. II. 224.

Strub, Emma,
I. Über Milchsterilisation.—C. f. B. 1890. VII. 665.—K. J. I. 89.

Strubell, A.,
I. Untersuchungen über den Bau und die Entwicklung des Rübennematoden, Heterodera Schachtii Schmidt.—Bibliotheca zoologica. Heft 2. Cassel. 1888. Th. Fischer.—C. f. B. 1887. I. 603. 1889. VI. 423.

Struve, Heinrich,
I. Über Kephir.—Ber. d. D. Chem. Ges. 1884. XVII. 314, 1364.

Strzyzowski, Casimir,
See Galli-Valerio und Strzyzowski.

Sturgis, W. C.,
I. Stem-rot.—Annual Report of the Connecticut Agricultural Experiment Station for 1891. New Haven. 1892, p. 184.
II. Preliminary Report on the so-called "Pole-burn" of Tobacco.—*Ibid.*, p. 168.

Stutzer, Adolf,
I. Über das Vorkommen von Nuclein in den Schimmelpilzen und in der Hefe.—Z. f. physiolog-Chemie. 1882. VI. 572.—Ch. C. 1882. 597.—Z. g. Br. 1882. V. 324.
See also Burri, Herfeldt und Stutzer; Burri und Stutzer.

Suchsland, E.,
I. Über Tabaksfermentation.—Ber. d. D. Bot. Ges. 1891. IX. 79.—C. f. B. 1892. XII. 723.—K. J. II. 230.
II. Über das Wesen der Tabakfermentation und über die sich daraus ergebende Möglichkeit, den Fermentationsprozess behufs Veredelung der Tabake zu beeinflussen.—Periodische Mitteilungen des Tabak-Vereins zu Mannheim. 1892. No. 38. 211.

SUGG,
See ERMENGEM und SUGG.
SVOBODA, A.,
See KLAUDI und SVOBODA.
SWAN, A. P.,
I. C. f. B. 2. Abt. 1896. II. 1.
SWAN, P.,
I. On the Resisting Vitality of the Spores of Bacillus megaterium to the Conditions of Dryness.—Annals of Botany. 1893. VII. 153.—K. J. IV. 110.
SYMMERS, W. St. Clair,
I. Preliminary Note on a New Chromogenic Micro-organism found in the Vesicles of Herpes labialis: Bacillus virdians. — British Medical Journal. 1891. No. 1615, p. 1252. — C. f. B. 1892. XII. 165.—K. J. III. 43.
II. Note on a Peculiar Movement of Certain Intracellular Particles in Yeast Cells.—Transactions of the British Institute of Preventive Medicine. London. 1898. I.—C. f. B. 1. Abt. 1898. XXIII. 794.

TACKE, Br.,
I. Über die Entwicklung von Stickstoff bei der Fäulnis.—Landw. Jahrbücher. 1887. XVI. 917.—C. f. B. 1888. III. 588.
TACKE, IMMENDORF, HESSENLAND, SCHÜTTE und MINSSEN,
I. Über das Verhalten der Bakterien der Leguminosenknöllchen gegen Ätzkalk. —Mitteilungen d. Ver. z. Förderung der Moorkultur im deutschen Reiche. 1895. XIII. 389.—C. f. B. 2. Abt. 1896. II. 161.—Ch. C. 1896. II. 252.
TAHARA u. KITAO,
I. Abst. in Chem. Centralbl. 1889. I. 732.
TAKAHASHI, T.,
I. Bull. College Agric. Tokyo. 1902. IV. 395.
TANRET, C.,
I. Action du nitrate d'ammoniaque sur l'Aspergillus niger. — C. R. 1896. CXXIII. 948.—Ch. C. 1897. I. 116. —K. J. VII. 42.
II. Action des nitrate, sulfate, chlorhydrate et phosphate d'ammoniaque sur l'Aspergillus niger. — Bulletin de la Société chimique de Paris. 1897. IIIe série. XVII. 914.—Ch. C. 1898. I. 71.
III. Recherches sur les champignons. —Bulletin de la Société chimique de Paris. 1897. IIIe série. XVII. 921. —Ch. C. 1898. I. 71.—K. J. VIII. 88.
IV. Sur un nouveau principe immédiat de l'ergot de seigle, l'ergostérine. —Journal de pharmacie et de chimie. 1889. (5.) XIX. 225.—Ch. C. 1889. I. 421.
V. C. R. 1888. CVI. 418.
VI. *Ibid.* 1902. CXXXIV. 1586.

TAPPEINER, H.,
I. Untersuchungen über die Gärung der Cellulose, insbesondere über deren Lösung im Darmkanale.—Zeitschrift f. Biologie. 1884. XX. 52.—Ch. C. 1884. 180.
TEICHERT,
I. Milchztg. 1903. XXXII. 785.
TELESNIN, L.,
I. C. f. B. 2. Abt. 1904. XII. 205.
TENSI, W.,
I. Der Johannis- und Stachelbeerwein und die Bereitung der übrigen Beerenweine. Stuttgart. Ulmer. 1*s.*
TERESCHTSCHENKO, N. A.,
See KUBAREW u. TERESCHTSCHENKO.
THAUSING, Julius,
I. Die Theorie und Praxis der Malzbereitung und Bierfabrikation. 5th Ed. 1898. Leipzig. Gebhardt. Price, with atlas, bound, 40*s.*
THENARD,
I. Ann. de Chimie. 1803. XLVI. 294.
THIERFELDER, Hans,
See FISCHER und THIERFELDER; GÜNTHER und THIERFELDER; NUTTAL und THIERFELDER.
THOM,
I. Journ. of Mycology. 1905. 117.
II. Bull. Bureau of Animal Industry, U.S. Dep. Agriculture. 1906.
THOMAS, Pierre,
I. Sur la nutrition azotée de la levure.— C. R. 1901. CXXXIII. 312.—Ch. C. 1901. II. 649.
II. C. R. 1903. CXXXVI. 1015.
III. *Ibid.* 1902. CXXXIV. 610.
THOMSON, Robert D.,
I. Über die Resultate der Brotgärung und über den nährenden Wert des Brotes und Mehles verschiedener Länder.—The London, Edinburgh and Dublin Philosophical Magazine. 1843, p. 321.— Journal f. prakt. Chemie. 1844. XXXI. 188.
THOMSON, Robert T.,
I. Über die Natur und die chemischen Wirkungen der Essigmutter.—Annalen der Chemie und Pharmacie. 1852. LXXXIII. 89.
THÖRNER, W.,
I. Untersuchung der Milch auf Tuberkelbazillen. — Chemiker Zeitg. 1892. XVI. 791.—Ch. C. 1892. II. 78.—K. J. III. 177.
THUDICHUM,
I. J. f. prakt. Chem. 1882. XXV. 19. 1896. LIII. 49.
THUMM, K.,
I. Beiträge zur Kenntnis der fluoreszierenden Bakterien.—Arbeiten aus d. Bakteriolog. Institute d. Techn. Hochschule zu Karlsruhe. I. 291.—C. f. B. 2. Abt. 1895. I. 586.—Ch. C. 1895. II. 876.

THYLMAN u. HILGER,
I. A. f. Hygiene. 1888. VIII. 451.

TIEGEL,
I. Über die fiebererregenden Eigenschaften des Microsporon septicum. Dissertation. Bern. 1871. — Correspondenzblatt f. schweizerische Ärzte. 1871. 275.

TIEGHEM, Philippe Edouard Léon van,
I. Sur les prétendus cils des Bactéries.— Bulletin de la Société Botanique de France. 1879. XXVI.
II. Traité de Botanique. 1883.
III. Observations sur les bactériacées vertes.—Bull. de la Société Bot. de France. 1880. XXVII. 174.
IV. Sur le Bacillus Amylobacter et son rôle dans la putréfaction des tissus végétaux.—Bull. de la Société Bot. de France. 1877. XXIV.
V. Identité du Bacillus Amylobacter et du Vibrion butyrique de Pasteur.—C. R. 1879. LXXXIX. 5.—Z. g. Br. 1879. II. 415.
VI. Sur la fermentation de la cellulose.— Bull. de la Société Bot. de France. 1879. XXVI. 28.—C. R. 1879. LXXXVIII. 205.
VII. Sur la gomme de sucrerie.—Annales des Sciences naturelles. Botanique. 6e série. 1878. VII. 180.
VIII. Sur la fermentation ammoniacale.— C. R. 1864. LVIII. 210.
IX. Action de la lumière sur la végétation du Penicillium glaucum dans l'huile.— Bull. de la Société Bot. de France. 1881. XXVIII. 186.
XII. Ann. d. Sci. Nat. Bot. 1867. VIII. 240.
XIII. C. R. 1867. LXV. 1091.—Ann. de l'Ecole Normale Supér. 1869. VI. 27.

TIEMANN, F., und GÄRTNER, A.,
I. Die chemische und mikroskopisch-bakteriologische Untersuchung des Wassers. 4th Ed. 1895. Braunschweig. Vieweg. 24*s.*, bound.

TIMM, H.,
I. Die Obst- und Gemüseverwertung für Haushaltungs- und Handelszwecke. Stuttgart. Ulmer. Boards, 3*s.* 8*d.*
II. Der Johannisbeerwein und die übrigen Obst- und Beerenweine. 3rd Ed. Stuttgart. 1896. E. Ulmer. 3*s.*, bound.

TIMPE, Hermann,
I. Über die Beziehungen der Phosphate und des Caseins zur Milchsäuregärung. —A. f. Hygiene. 1893. XVIII. 1.—C. f. B. 1894. XV. 425.—K. J. IV. 193.

TIRABOSCHI,
I. Annali di Botanica. 1905. II. 137.
II. *Ibid.* II. 150.

TISCHUTKIN, N.,
I. Die Rolle der Bakterien bei der Veränderung der Eiweissstoffe auf den Blättern von Pinguicula.—Ber. d. D. Bot. Ges. 1889. VII. 346.—C. f. B. 1890. VII. 288.
II. Über die Rolle der Mikro-organismen bei der Ernährung insektenfressender Pflanzen.—Acta horti petropol. 1892. XII. 1.—C. f. B. 1893. XIII. 134.— K. J. III. 57.

TIZZONI, Guido, u. CATTANI, G.,
I. Über die Widerstandsfähigkeit der Tetanusbacillen gegen physikalische und chemische Einwirkungen. — Archiv f. experimentelle Pathologie u. Pharmakologie. 1891. XXVIII. 41.—C. f. B. 1. Abt. 1891. IX. 487.

TODUR,
I. Contribution à l'étude de l'action des sels inorganiques et organiques d'argent sur diverses espèces d'Aspergillus. Nancy. 1905.

TOLLENS, B.,
I. Über eine Lampe zur Herstellung von Formaldehyd.—Ber. d. D. Chem. Ges. 1895. XXVIII. 261.—Ch. C. 1895. I. 729.
II. Kurzes Handbuch der Kohlenhydrate. I. Bd. 2nd Ed. 1898. II. Bd. 1895.
III. Über den Nachweis der Pentosen mittelst der Phloroglucin - Salzsäure-Absatzmethode.—Ber. d. D. Chem. Ges. 1896. XXIX. 1202.—Ch. C. 1896. II. 65.
IV. Ber. d. D. Chem. Ges. 1898. XXXI. 2150.
V. J. Fed. Inst. Brewing. 1898. IV. 438. — J. f. Landwirtschaft. 1901. XLIX. 29.
VI. Ber. d. D. Chem. Ges. 1885. XVIII. 26, 2611. — Liebig's Ann. 1886. CCXXXII. 169.
VII. Handbuch d. Kohlenhydrate. 1896. II. 186.
See also FEILITZEN und TOLLENS; STONE und TOLLENS.

TOLLENS, B., u. GLAUBITZ, H.,
I. Über den Pentosangehalt verschiedener Materialien, welche zur Ernährung dienen, und über den Verbleib des Pentosans bei den Operationen, welchen die obigen Materialien unterworfen werden. — Journal f. Landwirtschaft. 1897. XLV. 97.—Ch. C. 1897. I. 613.

TOLOMEI, Giulio,
I. Das Gerinnen der Milch in der Gewitterluft.—Milchzeitung. 1891. XX. 519. —K. J. II. 186.
II. Die Nitrifikation in Mauern.—Atti R. Accad. dei Lincei Roma. 1894. (5.) Vol. 3, p. 356.—Ch. C. 1894. I. 1115.— K. J. V. 265.
III. Einwirkung des Lichtes auf die Essiggärung.—Staz. sperim. agrar. ital. 1891. XX. 380.—Ch. C. 1891. II. 254. —K. J. II. 230.
IV. Einwirkung der Elektrizität auf die Essiggärung.—Staz. sperim. agrar. ital. 1891. XX. 380.—Ch. C. 1891. I. 458. —K. J. I. 139.

V. Über ein lösliches Ferment, welches sich im Weine findet.—Atti R. Accad. dei Lincei Roma. 1896. V. 52.—Ch. C. 1896. I. 777.—K. J. VII. 247.
VI. Über die Fermentation der Oliven und die Oxydation des Olivenöles.—Atti R. Accad. dei Lincei Roma. 1896. V. 122. — Ch. C. 1896. I. 879. — K. J. VII. 246.
VII. Einwirkung des Lichtes auf Saccharomyces ellipsoideus. — Accad. dei Lincei. Rend. 1892. (5.) I. 320.—K. J. III. 119.

Topf, G.,
I. Einige Beobachtungen über die Reinzucht und Beurteilung der Bierhefen.—Z. g. Br. 1888. XI. 285.

Trabut,
I. Bull. Soc. Bot. de France. 1895. XLII. 33.

Trapp, A.,
I. Die Methoden der Fleischkonservierung. Dissertation. Berlin. 1893.—K. J. IV. 105.
See also Plagge und Trapp.

Traube, Moritz,
I. Théorie der Fermentwirkungen. Berlin. 1858. F. Dümmler.—Poggendorff's Annalen. 1858. CLXXI. 331.
II. Über das Verhalten der Alkoholhefe in sauerstoffgasfreien Medien.—Ber. d. D. Chem. Ges. 1874. VII. 872, 1756.

Trécul, Auguste,
I. Sur le Bacillus Amylobacter. — C. R. 1865. LXI. 156, 436. 1867. LXV. 513.
II. Remarques sur des levures lactiques et alcooliques.—C. R. 1872. LXXV. 1160, 1169.

Treviranus, Ludolf Christian,
I. Über die Neigung der Hülsengewächse zu unterirdischer Knollenbildung.—Bot. Ztg. 1853. XI. 393.

Trevisan, Victor Graf,
I. Prospetto della Flora Euganea. Padova. 1842.

Tribondeau,
I. C. R. Soc. Biol. Bordeaux. 1901. LIII. 1905. LV. 104.

Trillat, A.,
I. Sur les propriétés antiseptiques de la formaldéhyde. — C. R. 1892. CXIV. 1278; CXV. 290.—Ch. C. 1892. I. 524; II. 224. 1893. I. 1047. 1894. II. 169, 857. 1896. I. 798.
II. Analyse qualitative et quantitative de la formaldéhyde.—C. R. 1893. CXVI. 891.—Ch. C. 1893. I. 1047.

Trombetta, Sergi,
I. Die Fäulnisbakterien und die Organe und das Blut ganz gesund getöteter Tiere.—C. f. B. 1891. X. 664.—Gives also the earlier bibliography.

Trommsdorf,
I. Tageblatt d. Frankfurter Naturforschersvers. 1867. 52.

Tscheppe, Ad.,
I. Gegorene Milch.—Pharmaceutical Journal. 1889, p. 66.—Ch. C. 1889. II. 457.

Tschirch, Alexander,
I. Beiträge zur Kenntnis der Wurzelknöllchen der Leguminosen.—Ber. d. D. Bot. Ges. 1887. V. 58.—C. f. B. 1887. 634.
II. Das Kupfer vom Standpunkte der gerichtlichen Chemie, Toxikologie und Hygiene. Mit besonderer Berücksichtigung der Reverdissage der Konserven und der Kupferung des Weins und der Kartoffeln. Stuttgart. 1893. F. Enke. —Gives numerous bibliographical references.
III. Angewandte Pflanzen - Anatomie. 1889. Wien u. Leipzig. I. Bd. 191.
IV. Microchemische Reaktionsmethoden im Dienste der techn. Mikroskopie.—Archiv d. Pharmacie. 1882. CCXX. 801.—Compare Bot. Ztg. 1883. XLI. 598.

Tsukamoto, M.,
I. Bull. Coll. Agric. Tokyo. 1897. II. 406.

Tubeuf, K. Freiherr von,
I. Pflanzenkrankheiten durch kryptogame Parasiten verursacht. Berlin. 1895. J. Springer. 16*s.*

Tulasne, Louis-René et Charles,
I. Fungi hypogæi. Paris. 1851.
II. Selecta fungorum carpologia. Paris. 1861.

Tumas, L.,
I. Über die Bedeutung der Bewegung für das Leben niederer Organismen.—Petersburger Medicin. Wochenschrift. 1882. No. 18.

Turpin,
I. Mémoire sur la cause et les effets de la fermentation alcoolique et acéteuse.—Mémoires de l'Académie d. sc. de l'Institut de France. 1840. XVII. 95.—C. R. 1838. VII. 369.

Turró, R.,
I. Contribucion ad estudio de la esporulacion de Bacillus anthracis. — Gaceta medica catalana. 1891. No. 3-4.—C. f. B. 1891. X. 91.—K. J. II. 74.

Udránsky,
I. Z. f. physiolog. Chemie. 1889. XIII. 539.

Uffelmann, J.,
I. Verdorbenes Brot.—C. f. B. 1890. VIII. 481.—K. J. I. 143.

Uhl,
I. Untersuchungen der Marktmilch in Giessen.—Z. f. Hygiene. 1892. XII. 475. —C. f. B. 1893. XIV. 67.—Ch. C. 1893. I. 168.—K. J. III. 176.

Ulpiani e Sarcoli,
I. Atti R. Accad. dei Lincei. 1902. XI. 173.

Ungaro, G.,
See Serafini und Ungaro.

Unna, P. G.,
I. Die Entwicklung der Bakterienfärbung —A reprint from C. f. B. 1888. III. 24. 1*s.* 6*d.*

Urech,
I. Ber. d. D. Chem. Ges. 1885. XVIII. 3048.
Ury, Jakob,
I. Über die Schwankungen des Bacterium coli commune in morphologischer und kultureller Beziehung. — Diss. Strassburg. 1894.—C. f. B. 1894. XVI. 579.
Uschinsky,
I. Über eine eiweissfreie Nährlösung für pathogene Bakterien nebst einigen Bemerkungen über Tetanusgift. — C. f. B. 1893. XIV. 316.
Uspenski, A. B.,
I. Zur Bakteriologie des Kwass. Dissert. St. Petersburg. 1891. Russian.
Uyeda,
I. The Bot. Mag. Tokyo. 1902. XV. 160.

Vandam, Léon,
I. Etude sur un bacille visqueux des bières anglaises: Bacillus viscosus III.—Bull. de l'Association belge des Chimistes. 1895. IX. 245.—W. f. Br. 1896. XIII. 31.—Z. g. Br. 1896. XIX. 161.
Vandevelde, A. J. J.,
I. Untersuchungen über Plasmolyse; Bestimmung der Giftigkeit von Alkoholen.—Handelingen van III. Vlammsch Natuuren Geneeskündig Congres. Antwerpen. 1899.—Ch. C. 1900. I. 481.
II. Bull. Assoc. belge des Chimistes. 1903. XVII. 398.
Vandevelde, G.,
I. Studien zur Chemie des Bacillus subtilis. —Z. f. physiolog. Chemie. 1884. VIII. 367.—Ch. C. 1884. 645.
Vanha, Johann, und Stoklasa, Julius,
I. Die Rüben-Nematoden (Heterodera, Dorylaimus und Tylenchus). Mit Anhang über die Enchytraeiden. Berlin. 1896. P. Parey.
Varry, G.,
I. Ann. de chim. et de phys. 1835. LX. 69.
Vassillière, E., Charvet et Gayon, U.,
I. Appareils à pasteuriser les Vins. Bordeaux. 1897. 5*s*. 6*d*.
Vaudin, L.,
I. Sur un élément d'erreur dans la recherche du riz ajouté à la farine de froment.—Journal de Pharm. et de Chimie. (6.) 1899. IX. 431.
Vaughan, Victor C.,
I. Preliminary Note on the Chemistry of Tyrotoxicon. — Medical News. 1887, p. 369.—C. f. B. 1887. II. 497.
II. Über die Anwesenheit von Tyrotoxicon in giftigem Eis und giftiger Milch und seine wahrscheinliche Beziehung zur Cholera infantum.—A. f. Hygiene. 1887. VII. 420.—C. f. B. 1888. III. 400.
Verbno Laszczynski, Boleslaw de,
I. Über das Vorkommen eines peptonisierenden Enzyms (Peptase) im Malz und Versuche zur Trennung der stickstoffhaltigen Bestandteile in Malz, Würze und Bier.—Z. g. Br. 1899. XXII. 71. —Ch. C. 1899. I. 698.
Vereinbarungen zur einheitlichen Untersuchung Beurteilung von Nahrungs- und Genussmitteln,
I. 1897. Heft 1, 7. 1899. Heft 2, 118.
II. *Ibid.* 1899. Heft 2, 118.
Vergnette-Lamotte, de,
I. Des effets de la chaleur pour la conservation et l'amélioration des vins.—C. R. 1865. LX. 895. 1869. LXIX. 693, 801, 1048.
Viala, Pierre,
I. Les maladies de la vigne. 3rd Ed. 1893. Coulet, Montpellier; G. Masson, Paris. 20*s*.—Zeitschrift f. Pflanzenkrankheiten. 1894. IV. 250.
Viala, P., et Ravaz, L.,
I. Sur les périthèces du Rot blanc de la vigne.—C. R. 1894. CXIX. 443. 1897. CXXIV. 105.—C. f. B. 2. Abt. 1895. I. 298. 1897. III. 601.
Vieth, P.,
I. Die Behandlung der aus Molkereien wegzugebenden Magermilch bei herrschender Maul- und Klauenseuche.—Milchzeitung. 1894. XXIII. 329.—C. f. B. 1894. XVI. 745.
Vignal, William,
I. Contribution à l'étude des Bactériacées. Le mesentericus vulgatus.—Paris. 1889. —C. f. B. 1890. VII. 61.
Villiers, A.,
I. Sur la fermentation de la fécule par l'action du ferment butyrique.—C. R. 1891. CXII. 435, 536; CXIII. 144.—C. f. B. 1891. X. 283.—K. J. II. 243.
Vincenzi, Livio,
I. Über die chemischen Bestandteile der Spaltpilze. — Z. f. physiolog. Chemie. 1887. XI. 181.—Ch. C. 1887. 243.
Vines, Sydney H.,
I. The Influence of Light upon the Growth of Unicellular Organisms.—Arb. d. Botan. Inst. in Würzburg. 1878. II. 133.
Virchow, Rudolf,
I. Die Sarcina.—Archiv f. patholog. Anatomie und Physiologie. 1847. I. 264.
Viron, L.,
I. Die Rolle der Schizophyten bei den Umsetzungen, welche sich in den "destillierten Wässern" abspielen.—Journal de Pharmacie et de Chimie. 1891. XXIII. 586.—Ch. C. 1891. II. 181.—K. J. II. 226.
Voelcker,
I. On the Composition of Cheese and on the Practical Mistakes in Cheese-making. —Journal of the Royal Agricultural Society of England. 1861. XXII. 61.
Voit, Fritz,
I. Das Verhalten der Galaktose beim Diabetiker. — Zeitschr. f. Biologie. 1893. XXIX. 147.—Ch. C. 1893. I. 364.

VORDERMAN, A. G.,
I. Analecta op Bromatologisch gebied.—Geneeskundig Tijdschrift voor Nederl.-Indie. 1893. XXXIII. No. 3, p. 369.
II. Geneesk. Tijdschrift voor Nederl.-Indïe. 1894. XXXIV. No. 5.

VRANCKEN, J.,
I. Les levures de fond ; leurs causes, leurs effets, moyens de les éviter.—Bulletin de l'association des anciens élèves de l'école de brasserie de Louvain. 1897. No. 4.

VRIES, Hugo de,
I. Over blauwe Kaas.—Maanbladder Hollandsche Maatschappy von Landbouw. 1887. No. 5.—Milchzeitung. 1888. XVII. 861.—C. f. B. 1889. V. 383.
II. Wachstumsgeschichte des roten Klees. —Landw. Jahrbücher. 1877. VI. 936.

VUILLEMIN, P.,
I. Arch. de Parasitologie. 1904. VIII. 540.
II. C. R. 1904. CXXXVIII. 1350.

VUYLSTEKE, Julius,
I. Ein Beitrag zur Entwicklungsgeschichte der Mischsaaten von Saccharomyceten. —Z. g. Br. 1888. XI. 537. 1889. XII. 1.—Annales de Micrographie. 1888. I. 193.—C. f. B. 1. Abt. 1889. V. 766.

WAGENER,
I. Österr. Monatschr. f. d. Orient. 1881. No. 12.

WAGER, Harold,
I. The Nucleus of the Yeast Plant.—Annals of Botany. 1898. XII. 499. Gives the antecedent bibliography.—C. f. B. 2. Abt. 1899. V. 225.—K. J. IX. 31.
II. On the Presence of Centrospheres in Fungi.—Annals of Botany. 1894. VIII. 321.

WAGNER, Paul,
I. Die geringe Ausnützung des Stallmist Stickstoffes und ihre Ursachen.—Deutsche landw. Presse. 1895. XXII. No. 11–14.—Chem.-Zeitg. Repert. 1895. XIX. 71.—Ch. C. 1895. I. 894.
II. Ber. d. D. Chem. Ges. 1888. XXI. 1238.

WAHL, G.,
I. Die Anzahl der Hefezellen im Bier.—Der Braumeister. 1889. 307.—Z. g. Br. 1889. XII. 297.

WAHL, R., und HANTKE, E.,
I. Über die Eiweisskörper in Bierwürze und Bier.—American Brewer's Review. 1894. VII. 492.—Ch. C. 1894. I. 788. —K. J. V. 135.

WAHL, Robert, und HENIUS, Max,
I. United States Patent (America) No. 540471 of 1895.

WAHRLICH, W.,
I. Bakteriologische Studien. I. : Zur Frage über den Bau der Bakterienzelle.—Scripta botanica horti bot. Petropolis. 1890–91. III. 30.—C. f. B. 1892. XI. 49.—K. J. II. 51.

WALKER, J. Wallace,
See PURDIE und WALKER.

WALLACE, Schippen,
I. Cases of Cheese-poisoning. — Medical News. 1887. II. 69.—C. f. B. 1887. II. 523.

WALTHER, P.,
I. Über Fick's Theorie der Labwirkung und Blutgerinnung. — Pflüger's Archiv. 1891. XXXVIII. 529.—K. J. II. 257.

WARBURG,
I. Untersuch. a. d. Bot. Inst. z. Tübingen. 1886. II. 54.

WARD, H. Marshall,
I. Experiments on the Action of Light on Bacillus anthracis.—Royal Society of London. 1892. December 15.—C. f. B. 1. Abt. 1893. XIII. 568.—K. J. III. 77 ; IV. 122.
II. The Ginger-beer Plant, and the Organisms composing it.—Philosophical Transactions. 1892. CLXXXIII. B. 125. With 6 plates. — Proceedings of the R. Society. 1892. L. No. 304-305.—C. f. B. 1892. XI. 689.—K. J. II. 133 ; III. 138.
III. On the Characters, or Marks, employed for classifying the Schizomycetes. — Annals of Botany. 1892, April.—C. f. B. 1. Abt. 1892. XII. 789.
IV. On the Biology of Bacillus ramosus (Fraenkel), a Schizomycete of the River Thames.—Fourth Report to the Royal Society Water Research Committee.—Proceedings of the R. Society. 1895. LVIII. 1.—Bot. Ztg. 2. Abt. 1896. LIV. 119.
V. On the Tubercular Swellings on the Roots of Vicia faba. — Philosophical Transactions. 1887. CLXXVIII. 539.—Biedermann's Centralblatt. 1887. XVI. 787.—Ch. C. 1888. 113.
VI. A Lily Disease.—Annals of Botany. 1888. II. 319.—C. f. B. 1889. V. 842. — Bot. Ztg. 1889. XXXXVII. 306.
VII. Further Experiments on the Action of Light on Bacillus anthracis.—Communic. made to the Royal Society. 1893.—C. f. B. 1. Abt. 1894. XV. 1019.—Ch. C. 1893. II. 61.—K. J. IV. 122 ; V. 100.
VIII. The Brewer's Guardian.—Abst. in W. f. Br. 1892. XIX. 75.
IX. Proc. Roy. Soc. 1892. L. Nos. 304 and 305.

WARINGTON, Robert,
I. The Chemical Actions of Some Micro-organisms. London. 1888.—Chem. News. 1888. LVII. 346.—C. f. B. 1889. VI. 498.—Ch. C. 1888. 1034.
II. Curdling of Milk by Micro-organisms.—The Lancet. 1888. I. No. 25.—Journal of the Chemical Society. 1888. LIII. 727.—C. f. B. 1888. IV. 394.

III. On Nitrification. — Chemical News. 1891. LXIII. 296.—Ch. C. 1890. I. 774. 1891. II. 202.—K. J. III. 215.

WARMING, Eugen,
I. Om nogle ved Danmarks Kyster levende Bacterier. — Videnskabelige Meddelelser fra den naturhistoriske Forening i Kjöbenhavn. 1875. Nos. 20 to 28.

WARSCHAWSKY, J.,
I. C. f. B. 2. Abt. 1904. XII. 400.

WASSERZUG, E.,
I. Variations de forme chez les bactéries. — Ann. Inst. Past. 1888. II. Nos. 2 and 3.—C. f. B. 1. Abt. 1888. III. 783.
II. Sur les spores chez les levures.—Bulletin de la Société botanique de France. 1888. XXXV.—C. f. B. 1. Abt. 1888. IV. 232.

WATSON, D.,
I. Engl. Patent No. 22846 of 1897.

WEBSTER, W.,
I. Reinigung der Abwässer und Schmutzwässer.—Ch. C. 1891. I. 336.

WEGENER,
I. Verfahren zur Nutzbarmachung von Hefe.—K. J. IX. 104.

WEGNER,
I. Über Dextran.—Zeitschrift f. Zuckerindustrie. 1890. XL. 789.—Ch. C. 1890. II. 1000.

WEHMER, C. (Karl),
I. Zur Zersetzung der Oxalsäure durch Licht- und Stoffwechselwirkung.—Ber. d. D. Bot. Ges. 1891. IX. 218.—Ch. C. 1891. II. 665.—K. J. II. 233.
II. Durch Botrytis hervorgerufene Blattfäule von Zimmerpflanzen, nebst einigen kritischen Bemerkungen zur Speciesfrage. — Zeitschrift f. Pflanzenkrankheiten. 1894. IV. 204.
III. Beiträge zur Kenntnis einheimischer Pilze. Erstes Heft. 1893. Hannover. Hahn.—Ch. C. 1893. II. 457.—K. J. IV. 241, 268.
IV. *Ibid.* Zweites Heft. 1895. Jena. G. Fischer.—Bot. Ztg. 2. Abt. 1895. LIII. 377. 1896. LIV. 9, 44.—C. f. B. 2. Abt. 1897. III. 434.—K. J. VI. 72.
V. Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze.—Bot. Ztg. 1891. XLIX. 233.—Z. g. Br. 1891. XIV. 283.—K. J. II. 110.
VI. Zur Oxalsäuregärung durch Aspergillus niger.—C. f. B. 2. Abt. 1897. III. 102.—Ch. C. 1897. I. 768. — K. J. VIII. 234.
VII. Über die Wirkung einiger Gifte auf Hefe u. Gärung.—Chemiker-Ztg. 1899. XXIII. 163.—C. f. B. 2. Abt. 1899. V. 236.—Ch. C. 1899. I. 795.—K. J. X. 160.
VIII. Aspergillus oryzæ, der Pilz der japanischen Sake-Brauerei.—C. f. B. 2. Abt. 1895. I. 150.—K. J. VI. 315.
IX. Einige Beobachtungen über den Einfluss des Alters und der Temperatur auf die Entwicklungsfähigkeit von Mycelpilzsporen.—C. f. B. 2. Abt. 1897. III. 104.—Ch. C. 1897. I. 996.
X. Über zwei weitere, freie Citronensäure bildende Pilze.—Chemikerzeitung. 1897. XXI. 1022.—Ch. C. 1898. I. 269.—K. J. VIII. 235.
XI. Die chinesische Hefe und der sogen. Amylomyces (= Mucor Rouxii).—C. f. B. 2. Abt. 1900. VI. 353. 1901. VII. 599.—Ch. C. 1900. II. 55.
XII. Über die Verflüssigung der Gelatine durch Pilze. — Chemiker-Ztg. 1895. XIX. 2038.—C. f. B. 2. Abt. 1896. II. 92.—Ch. C. 1896. I. 49.—K. J. VI. 73.
XIII. Der javanische Ragi und seine Pilze. I.—C. f. B. 2. Abt. 1900. VI. 610.
XIV. *Ibid.* II.—*Ibid.* 2. Abt. 1901. VII. 313.
XV. Chem. Ztg. 1897. XXI. 73.
XVI. *Ibid.* 1902. XXVI. No. 22.
XVII. Die Pilzgattung Aspergillus Genf. 1901.
XVIII. C. f. B. 1. Abt. 1903. XXXV. 140.
XIX. *Ibid.* 2. Abt. 1896. II. 140.
XX. Ber. d. D. Bot. Ges. 1893. XI. 499.
XXI. C. f. B. 2. Abt. 1897. III. 149.
XXII. Hedwigia. 1894. XXXIII. 211.
XXIII. Beiträge z. Kenntnis einheimischer Pilze. 1896. Heft 2. 73–77.
XXIV. *Ibid.* 68.
XXV. Ber. d. D. Bot. Ges. 1904. XXII. 476. *See also* XXI.
XXVI. *Ibid.* 1891. IX. 223.
XXVI*a*. *Ibid.*, p. 163.
XXVII. Liebig's Ann. 1892. CCLXIX. 383.
XXVIII. Beiträge z. Kenntnis einheimischer Pilze. Hannover u. Leipzig. 1892. Heft 1.—Sitzgsber. d. K. Akad. Wiss. Berlin. 1893. 519.
XXIX. Chem. Ztg. 1897. XXI. 1022.
XXX. Bull. Soc. Chimique. 1893. 728.—C. R. 1893. CXVII. 332.
XXXI. Abhandlungen d. D. Seefischereivereins. 1898. III. 1.
XXXII. C. f. B. 2. Abt. 1905. XIV. 682.
XXXIII. Bot. Ztg. 2. Abt. 1898. LVI. 53.
XXXIV. Beiträge z. Kenntnis einheimischer Pilze. 1896. Heft 2, p. 70.

WEIBEL, E.,
I. Untersuchungen über Vibrionen.—C. f. B. 1887. II. 465. 1888. IV. 225.

WEIBEL, L.,
See BUNGENER und WEIBEL.

WEIDENBAUM, A.,
I. Über die morphologischen und physiologischen Unterschiede zwischen Oidium albicans und Oidium lactis.—Arbeiten d. St. Petersburger Naturforscher-Gesellschaft. Abt. f. Botanik. 1891. 26.—C. f. B. 1. Abt. 1892. XI. 569.

WEIDENBAUM, Josef,
I. Zur quantitativen Bestimmung des Glycogens. — Pflüger's Archiv. 1899. LXXV. 113.—Ch. C. 1899. I. 1167.

WEIDMANN,
I. Untersuchungen über die Zusammensetzung und den Reifungsprozess des Emmenthaler Käses. — Landw. Jahrbücher. 1882. XI. 587.

WEIGMANN, Heinrich,
I. Über den jetzigen Stand der bakteriolog. Forschung auf dem Gebiete des Käsereifungsprozesses.—C. f B. 2. Abt. 1896. II. 150.
II. Die Methoden der Milchkonservierung speziell das Pasteurisieren und Sterilisieren der Milch. With 22 Figs. Bremen. 1893. Heinsius.
III. Die Säuerung des Rahmes mittelst Bakterien-Reinkulturen.—Landw. Wochenbl. f. Schleswig-Holstein. 1890. No. 29.—C. f. B. 1892. XI. 762.
IV. Neue Mitteilungen über Rahmsäuerung mittelst Reinkulturen von Säurebakterien.—Milchzeitung. 1890. XIX. 945.—C. f. B. 1892. XI. 762.
V. Erfahrungen über die Rahmsäuerung mit Bakterien-Reinkulturen.—Landw. Wochenbl. f. Schleswig-Holstein. 1892. No. 16.—C. f. B. 1892. XI. 762.
VI. Die Bakteriologie im Dienste der Milchwirtschaft. — Milchzeitung. 1891. XX. 227.
VII. Der Organismus der sogenannten langen Wei. — Milchzeitung. 1889. XVIII. 982.—Ch. C. 1890. I. 431.
VIII. Über die Lochbildung und Bläh-ung der Käse.—Milchzeitung. 1890. XIX. 741.—K. J. I. 92.
IX. Über bittere Milch.—Milchzeitung. 1890. XIX. 881.—K. J. I. 88.
X. Über den Anteil der Milchsäurebakterien an der Reifung der Käse.—C. f. B. 2. Abt. 1899. V. 630.—Ch. C. 1899. II. 724.
XI. Milchztg. 1890. XIX. 743.

WEIGMANN, H., und ZIRN, G.,
I. Über das Verhalten der Cholerabakterien im Käse.—C. f. B. 1894. XV. 286.—Ch. C. 1894. I. 968.—K. J. V. 225.
II. Über seifige Milch.—C. f. B. 1894. XV. 463.—Ch. C. 1894. I. 166.—K. J. V. 215.

WEINLAND,
I. Z. f. Biologie. 1901. XXXVIII. 16, 606.

WEINLAND, E.,
I. Z. f. Biologie. 1901. XLII. 55. 1902. XLIII. 86.

WEIS, Fr.,
I. Über das proteolytische und ein eiweisscoagulierendes Enzym in keimender Gerste (Malz).—Z. f. physiolog. Chemie. 1900. XXXI. 79.—Ch. C. 1901. I. 56.

WELEMINSKY, Friedrich,
I. Über Sporenbildung bei Dematium pullulans de Bary.—C. f. B. 2. Abt. 1899. V. 297.

WELLER, H.,
I. Über das Vorkommen von Alkohol in der Milch.—Forschungsberichte ü. Lebensmittel u. ihre Beziehungen z. Hygiene, etc. 1897. IV. 206.—Ch. C. 1897. II. 527.

WELLS, J. G.,
See MORRIS and WELLS.

WELTE, Eugen,
I. Über das Verschimmeln des Brotes. —A. f. Hygiene. 1895. XXIV. 84; XXV. 104. — Ch. C. 1895. II. 543. 1896. I. 54.—K. J. VI. 301.

WENT, C., und PRINSEN GEERLIGS, C.,
I. Over suiker en alcoholvorming door organismen in verband met de verwerking der naproducten in de rietsuikerfabriken.—Mededeelingen van het proefstation voor suikerriet in West-Java te Kagok-Tegal. 1895. — Verhandeling. d. Koninkl. Akad. v. Wetenschappen te Amsterdam. 1895. Ser. II. Deel 4. No. 2.—C. f. B. 2. Abt. 1895. I. 501, 504.—Ch. C. 1894. II. 633.—K. J. V. 152.
II. Verh. d. K. Akad. Wetenschappen te Amsterdam. 1894. Ser. II. IV. No. 2.
III. Die deutsche Zuckerindustrie. 1894. XIX. 1043.

WENT, F. A. F. C.,
I. Ann. d. Sci. Nat. Bot. 7th Series. I.
II. Verhandl. d. Koninkl. Akad. Wetensch. Amsterdam. 1904. Aug. 26, p. 83.
III. Recueil des travaux bot. Néerland. 1904. No. 1.—Abst. in W. f. Br. 1904. XXI. 698.
IV. C. f. B. 2. Abt. 1901. VII. 544.
V. Jahrb. wiss. Bot. 1901. XXXVI. 611.

WERMISCHEFF,
I. Recherches sur les microbes acétifiants. —Ann. Inst. Past. 1893. VII. 213.—K. J. IV. 248.

WERNER, C.,
I. Die Bedingungen der Konidienbildung bei einigen Pilzen. Dissert. 1898.—C. f. B. 2. Abt. 1899. V. 289.

WERNKE,
I. Dissert. Dorpat. 1879. Quoted by WILL, I.

WESENBURG, G.,
I. C. f. B. 2. Abt. 1902. VIII. 627.

WEYL, Theodor,
See KITASATO und WEYL; PICTET und WEYL.

WICHMANN, Heinrich,
I. Über Wasserfiltration.—Mitteilungen d. Österr. Versuchs-Station f. Brauerei und Mälzerei in Wien. 1892. Heft V. —C. f. B. 1893. XIII. 22.—K. J. III. 73.

II. Biologische Untersuchung des Wassers für Brauereizwecke. — Mitteilungen d. Österr. Versuchs-Station für Brauerei und Mälzerei. 1892. V.—C. f. B. 1893. XIII. 207.—K. J. III. 134.

III. Über die Ascosporenzüchtung auf Thon.—C. f. B. 1. Abt. 1893. XIV. 62. —K. J. IV. 39.

IV. Neuere Hefereinzucht-Apparate. — Mitteilungen d. Österr. Versuchs-Station f. Brauerei, etc. 1894. VI. 39. 1902. X. 3.—K. J. V. 189.

See also Rohn und Wichmann.

Wiener, Emil,

See Gruber und Wiener.

Wierigo,

I. Sitzungsberichte des Odessaer naturw. Vereines. 1886, 1887.—Wratsch. 1886. 352. 1887. 576. Russian.

Wiesner, Julius,

I. Die heliotropischen Erscheinungen im Pflanzenreiche.—Denkschriften der Wiener Akademie. Mathem.-naturw. Klasse. 1879. XXXIX. Abth. 1. 143-209. 1882. XLIII. Abth. 1. 1-92.

II. Untersuchungen über den Einfluss, welchen Zufuhr und Entziehung von Wasser auf die Lebentätigkeit der Hefezellen äussern. — Sitzungsber. d. Akademie d. Wissenschaften in Wien. Mathem.-naturw. Klasse. 1869. LIX. 2. Abt. p. 495.

Wigand, Alb.,

I. Entstehung und Fermentwirkung der Bakterien.—Marburg. 1884. Elwert.

II. Das Protoplasma als Ferment organismus. Marburg. 1888.

Wiggers, H. A. L.,

I. Untersuchung über das Mutterkorn. Annalen der Pharmacie. 1832. I. 129.

Wijsmann, H. P.,

I. De diastase beschouwed als mengsel van Maltase en Dextrinase. Amsterdam. 1889. Spin & Co.—Recueil des travaux chimiques des Pays-bas. 1890. IX. 1. —Z. g. Br. 1890. XIII. 186.

II. Über den Stickstoffgehalt von Saccharomyces ellipsoides.—Z. g. Br. 1891. XIV. 381.—Ch. C. 1891. II. 759.—K. J. II. 120.

Wilckens, Martin,

See Adametz u. Wilckens.

Wildiers, E.,

I. Nouvelle substance indispensable au développement de la levure.—La Cellule. 1901. XVIII. 313.

Wiley,

I. Journ. Franklin Inst. 1897. CXLIII. 293.

Wilfarth, H.,

I. Über die Stickstoff-Aufnahme der Pflanzen.—Tageblatt der Naturforscher-Versammlung zu Bremen. 1890.—K. J. I. 131.

Wilhelm, C.,

I. Beiträge z. Kenntnis d. Pilzgattung Aspergillus. Dissert. Berlin. 1877. 36. —Bot. Zeitung. 1881. XXXIX. 534.

Wilhelmi, Armand,

I. Beiträge zur Kenntnis des Saccharomyces guttulatus.—C. f. B. 2. Abt. 1898. IV. 305. Gives the antecedent bibliography.—K. J. IX. 34.

Will, Heinrich,

I. Über die Wirkung einiger Desinfektionsmittel auf Hefe.—Z. g. Br. 1893. XVI. 151 *et seq.*, 411. 1894. XVII. 43. Has many references to the older literature. —Ch. C. 1893. II. 60. 1894. I. 159, 964.—K. J. IV. 171; V. 201.

II. Untersuchungen über die Verunreinigungen gebrauchter Trubsäcke. — Z. g. Br. 1892. XV. 77.—C. f. B. XII. 148.—K. J. III. 166.

III. Ein Beitrag zur Kenntnis der sogen. Glutinkörperchen in der Würze, im Bier und in der Hefe.—Z. g. Br. 1894. XVII. 187. 1897. XX. 77.—Ch. C. 1894. II. 392.

IV. Die braun gefärbten Ausscheidungen (Hopfenharz-Ausscheidungen), welche der Bierhefe beigemengt sind; deren Bau im normalen und abnormalen Zustand, sowie deren Beschaffenheit.— Z. g. Br. 1894. XVII. 315.

V. Bemerkung zu einer Arbeit von Paul Lindner (VII.).—Z. g. Br. 1896. XIX. 675.

VI. Notiz betreffend den Nachweis von wilden Hefenarten in Brauereihefen und Jungbieren sowie das Vorkommen von Saccharomyces apiculatus in denselben. —Z. g. Br. 1893. XVI. 29.—Ch. C. 1893. I. 547; II. 689.—K. J. IV. 170.

VII. Über einen ungeformten Eiweisskörper, welcher der untergärigen Bierhefe beigemengt ist, und dessen Beziehung zu dem sogen. gelatinösen Netzwerk, welches beim Eintrocknen der Bierhefe entsteht, nebst einigen Beobachtungen über Netzbildung in der Kahmhaut.—Z. g. Br. 1897. XX. 447.—C. f. B. 2. Abt. 1898. IV. 130.—Ch. C. 1897. II. 869.

VIII. Vergleichende Untersuchungen an vier untergärigen Arten von Bierhefe. Teil I. bis V.—Z. g. Br. 1895. XVIII. 1, 217.—C. f. B. 2. Abt. 1895. I. 449. 1896. II. 752. 1898. IV. 367.

IX. Zwei Hefearten, welche abnorme Veränderungen im Bier veranlassen.—Z. g. Br. 1891. XIV. 145.—C. f. B. 1891. X. 521.—Ch. C. 1891. II. 353. This gives a figure.—K. J. II. 150.

X. Die Hefenzelle, deren Aussehen und Beschaffenheit in den verschiedenen Stadien der Entwicklung und des Zerfalles unter dem Mikroskop.—Allgem. Brauer- und Hopfen-Zeitung. 1892. XXXII. 1088.

XI. Schielige Biere. Bemerkungen zu einer Arbeit von C. J. Lintner (IV.).—Z. g. Br. 1891. XIV. 81.—K. J. II. 149.
XII. Studien über die Proteolyse durch Hefen. I. u. II.—Z. g. Br. 1898. XXI. 139. 1901. XXIV. 113.—C. f. B. 2. Abt. 1898. IV. 753. 1901. VII. 794. —Ch. C. 1898. I. 1141.
XIII. Eine Mycoderma-Art und deren Einfluss auf Bier.—Z. g. Br. 1899. XXII. 391. 1900. XXIII. 185.—C. f. B. 2. Abt. 1899. V. 842. 1900. VI. 561.—Ch. C. 1899. II. 449.—K. J. X. 153.
XIV. Gerbstoff - Reaktionen an Hefezellen und deren Beimengungen aus gehopfter Würze.—Z. g. Br. 1900. XXIII. 325.—C. f. B. 2. Abt. 1900. VI. 807.
XV. Vergleichende Untersuchungen an vier untergärigen Arten von Bierhefe. Teil VI.—Z. g. Br. 1898. XXI. 443. 1899. XXII. 151. 1902. XXV. 241. —C. f. B. 2. Abt. 1899. V. 726. 1902. IX. 135.—K. J. IX. 86; X. 93.
XVI. Untersuchungen über das Ausarten der Brauereihefe.—Z. g. Br. 1898. XXI. 243.—C. f. B. 2. Abt. 1898. IV. 808.—K. J. IX. 91.
XVII. Über Sporen- und Kahmhautbildung bei Unterhefe.—Z. g. Br. 1887. X. 357.—C. f. B. 1. Abt. 1887. II. 592.
XVIII. Über die mechanische Bierklärung durch Späne und Filtration und ihren Einfluss auf die Qualität und Haltbarkeit des Bieres. Vortrag.—Z. g. Br. 1896. XIX. 616.—K. J. VII. 113.
XIX. Welche Erfahrungen sind mit der Reinhefe im Brauerei betrieb gemacht worden? Vortrag.—Z. g. Br. 1897. XX. 591. K. J. VIII. 110.
XX. Zur Frage der alkoholischen Gärung ohne Hefezellen.—Z. g. Br. 1898. XXI. 291.—C. f. B. 2. Abt. 1899. V. 195.—Ch. C. 1898. II. 439.—K. J. IX. 318.
XXI. Einige Beobachtungen über die Lebensdauer getrockneter Hefe.—Z. g. Br. 1896. XIX. 453. 1897. XX. 91. 1898. XXI. 75. 1899. XXII. 43. 1900. XXIII. 11. 1901. XXIV. 3. 1902. XXV. 49. 1903. XXVI. 57.—C. f. B. 2. Abt. 1897. III. 17. 1898. IV. 485. 1899. V. 527. 1900. VI. 226. 1901. VII. 438. 1902. IX. 69. 1903. X. 251. —K. J. VII. 106; VIII. 90; IX. 85; X. 172; XI. 127.
XXII. Die Farbe des Bieres unddie Hefe. —Z. g. Br. 1901. XXIV. 501.—Ch. C. 1901. II. 714.
XXIII. Hefewasser zur biologischen Analyse.—Z. g. Br. 1901. XXIV. 289. —C. f. B. 2. Abt. 1901. VII. 892. —Ch. C. 1901. II. 139.
XXIV. Die Methoden, welche bei der Reinzüchtung von Hefe und ähnlichen Organismen durch Einzellkultur auf festen Nährböden zur Feststellung der Lage der ausgewählten Zellen in den Kulturen zur Anwendung kommen.—C. f. B. 2. Abt. 1896. II. 483.—K. J. VII. 8.
XXV. Z. g. Br. 1900. XXIII. 748.
XXVI. *Ibid.* 1898. XXI. 307.
XXVII. *Ibid.* 1902. XXV. 33.
XXVIII. *Ibid.* 1895. XVIII. 249.
XXIX. *Ibid.* 1897. XX. 59.
XXX. *Ibid.* 1895. XVIII. 1. 1898. XXI. 1899. XXII. 1902. XXV. 1904. XXVII.
XXXI. *Ibid.* 1903. XXVI. 283.
XXXII. *Ibid.*, p. 284.
XXXIII. *Ibid.* 1905. XXVIII. 128.
XXXIV. *Ibid.* 1896. 453.
XXXV. *Ibid.* 1897. XX. 363.
XXXVI. *Ibid.* 1896. XIX. 20.
XXXVII. *Ibid.* 1898. XXI. 139. 1901. XXIV. 113.

WILL, H., u. WANDERSCHECK,
I. Z. g. Br. 1906. XXIX. 73.

WILLCOX, W. H.,
See WOOD and WILLCOX.

WILLIAMS,
I. Chinese Commercial Guide. 4th Ed. 1856.

WILM,
I. Über die Einwanderung von Cholera-vibrionen ins Hühnerei.—A. f. Hygiene. 1895. XXIII. 145.—C. f. B. 2. Abt. XVII. 196, 892.—Ch. C. 1895. II. 171.

WILSON,
I. Über Atmung der Pflanzen. — Flora. 1882. LXXV. 93.

WINDISCH, K.,
I. Notice in Chem. Ztg. 1901. XXV. 240.
II. *Ibid.* 1902. XXVI. 865.
III. Arb. Kais. Ges. Amt. 1892. VIII. 175.
IV. *Ibid.* 1895. XI. 285.
V. Die Chem. Untersuchung u. Beurtheilung d. Weines. 1896. 42, 43.
VI. *Ibid.* 1896. 100.

WINDISCH, Wilhelm,
I. Sterilisierung von Kellern, Tennen, Fässern, etc., mittelst Dämpfen von Formaldehyd, sowie das Verhalten von Formaldehyd gegen Hefen und Bakterien.—W. f. Br. 1894. XI. 1531.—Ch. C. 1895. I. 276.—K. J. V. 95.
II. Über die Bildung und den Verbleib des Furfurols, sowie dessen Bedeutung im Brauereibetriebe.—W. f. Br. 1898. XV. 189.—Ch. C. 1898. I. 1213.—K. J. IX. 161.
III. Wie kommen die unedlen faden Geschmacksstoffe beim Maischprozess ins Bier.—W. f. Br. 1897. XIV. 393.
IV. W. f. Br. 1899. XVI. 653.
V. *Ibid.* XVI. 72.
VI. *Ibid.* 1895. XII. 655.
VII. *Ibid.* 1900. XVII. 91.

WINDISCH, W., und SCHELLHORN, B.,
I. Über das eiweissspaltende Enzym der gekeimten Gerste.—W. f. Br. 1900. XVII. 334.—Ch. C. 1900. II. 489.

WINKLER, Willibald,
I. Zur Charakterisierung der Duclaux'schen Tyrothrix-Arten, sowie über die Variabilität derselben und den Zusammenhang der peptonisierenden und Milchsäure-Bakterien.—C. f. B. 2. Abt. 1895. I. 609.—Ch. C. 1895. II. 1050.—K. J. VI. 250.

WINKLER, W.,
I. C. f. B. 2. Abt. 1902. VIII. 721.
II. Jahres. f. prak. Pharmazie. XXVI. 209.

WINOGRADSKY, Sergius,
I. Sur le rouissage du lin et son agent microbien.—C. R. 1895. CXXI. 742. —C. f. B. 2. Abt. 1896. II. 273.—Ch. C. 1896. I. 50.
II. Sur l'assimilation de l'azote gazeux de l'atmosphère par les microbes.—C. R. 1893. CXVI. 1385. 1894. CXVIII. 353.—Ch. C. 1893. II. 283. 1894. I. 590.—C. f. B. 1894. XVI. 129.—K. J. IV. 231; V. 255.
III. Recherches sur l'assimilation du nitrogène libre de l'atmosphère par les microbes.—Archives des sciences biologiques de St. Pétersbourg. 1895. III. 295.—Ch. C. 1895. I. 1123.—Revue mycologique. 1897, p. 95.—K. J. VI. 273.
IV. Über Eisenbakterien.—Bot. Ztg. 1888. XXXXVI. 261.—C. f. B. 1888. IV. 65. —Ch. C. 1888. 1035.
V. Beiträge zur Morphologie und Physiologie der Bakterien. I. Zur Morphologie und Physiologie der Schwefelbakterien. 1888. Leipzig. A. Felix. — C. f. B. 1889. V. 57.
VI. Über Schwefelbakterien.—Bot. Ztg. 1887. XXXXV. 489.—C. f. B. 1887. II. 590.—Ch. C. 1888. S. 1034. 1890. I. 1062.
VII. Recherches sur les organismes de la nitrification. I.-IV.—Ann. Inst. Past. 1890. IV. 213, 257, 760. 1891. V. 92. —C. R. 1890. CX. 1013.—C. f. B. 1890. VIII. 175 u. 392. 1891. IX. 351 u. 603.—Ch. C. 1890. I. 1061; II. 110. 1891. I. 327; II. 491. 1892. I. 216.—K. J. I. 101; II. 210.
VIII. Contributions à la morphologie des organismes de la nitrification.—Archives des sciences biologiques publ. par l'Inst. Imp. de médec. expérim. à St. Pétersbourg. 1892. I. 87.—C. f. B. 2. Abt. 1895. I. 243.—K. J. III. 221.
IX. Sur la formation et l'oxydation des nitrites pendant la nitrification.—C. R. 1891. CXIII. 89.—K. J. II. 212.
X. Zur Mikrobiologie des Nitrifikationsprozesses.—C. f. B. 2. Abt. 1896. II. 415.—Ch. C. 1896. II. 636.—K. J. VII. 209.
XI. Über die Wirkung äusserer Einflüsse auf die Entwicklung von Mycoderma vini. — Arbeiten d. St. Petersburger naturforsch. Gesellschaft. 1884. XIV. 132.—Botan. Centralblatt. 1884. XX. 165.

WINTER, Georg,
I. Heliotropismus bei Peziza Fuckeliana de By. — Bot. Ztg. 1874. XXXII. 1. It has some bibliographical references.
II. Die Pilze Deutschlands, Österreichs und der Schweiz. I. Abt.: Schizomyceten, Saccharomyceten und Basidiomyceten. Leipzig. 1884.
III. *Ibid.* II. Abt.: Gymnoasceen und Pyrenomyceten. Leipzig. 1887.
IV. In Rabenhorst's Kryptogamenflora Deutschlands. 2nd Ed. 1887. I. 2. Abt. 48, 61.

WINTER, H.,
I. Untersuchungen über das Zuckerrohr. Dissert. Halle. 1891. — Mededeelingen van het Proefstation Midden Java. Semarang 1890.

WINTERSTEIN, E.,
I. Zur Kenntnis der Pilzcellulose.—Ber. d. D. Bot. Ges. 1893. XI. 441. 1895. XIII. 65.—Ch. C. 1893. II. 756. 1895. I. 962.
II. Die in den Membranen der Pilze enthaltenen Bestandteile.—Z. f. physiolog. Chemie. 1894. XIX. 521; XX. 342. —Ch. C. 1894. II. 524. 1895. I. 280.
III. Über ein stickstoffhaltiges Spaltungsprodukt der Pilzcellulose.—Ber. d. D. Chem. Ges. 1894. XXVII. 3113, 3508. 1895. XXVIII. 167.—Ch. C. 1895. I. 29, 392, 690.—C. f. B. 2. Abt. 1895. I. 500.
IV. Zur Kenntnis der in den Membranen der Pilze enthaltenen Bestandteile. II. —Z. f. physiolog. Chemie. 1896. XXI. 134.—Ch. C. 1896. I. 114.

WIRGIN, G.,
I. Z. f. Hygiene. 1902. XL. 307.

WISSELINGH, C. van,
I. Mikrochemische Untersuchungen über die Zellwände der Fungi.—Pringsheim's Jahrbücher. 1898. XXXI. 619.—C. f. B. 2. Abt. 1899. V. 193.

WITTLIN, J.,
I. Über die angebliche Umänderung von Tyrothrix tenuis (Duclaux) in ein Milchsäure-Bacterium.—C. f. B. 2. Abt. 1896. II. 475.—K. J. VII. 193.

WLADIKA, Julius,
I. Zur Kenntnis der organischen Säuren in Fichtenbrühen.—Der. Gerber. 1890. No. 368.

WLADIMIROFF, Alexander,
I. Biologische Studien an Bakterien. I. Über das Verhalten beweglicher Bakterien in Lösungen von Neutralsalzen.—Z. f. Hygiene. 1891. X. 89.—K. J. II. 66

II. *Ibid.* II. Osmotische Versuche an lebenden Bakterien.—Zeitschrift f. physikalische Chemie. 1891. VII. 524.—K. J. II. 66.

WOHL,
I. Ber. d. D. Chem. Ges. 1898. XXXI. 1796.

WOLFF,
I. C. R. 1900. CXXXI. 1323.

WOLFF, Emil,
I. Beiträge zur Theorie und Praxis der Düngung. A reprint from the 12th ed. of Praktischen Düngerlehre. Berlin. 1892. Parey.

WOLFFHÜGEL, G.,
I. Über den Wert der schwefligen Säure als Desinfektionsmittel.—Mitteil. a. d. K. Gesundheits-Amte. 1881. I. 1888.—Ch. C. 1882. 334.
See also KOCH und WOLFFHÜGEL.

WOLFFIN, Alexander,
I. Hygienische Studien über Mehl und Brot. —A. f. Hygiene. 1894. XXI. 268.—Ch. C. 1894. II. 895.—K. J. VI. 300.

WOLLNY, R.,
I. Auf kaltem Wege sterilisierte eiweisshaltige Nährböden.—C. f. B 1892. XI. 752.—K. J. III. 17.

WOLTERING, P., und SASSEN, A.,
I. Über Reinigung von eisenhaltigem Grundwasser.—Nederl. Tijdschr. Pharm. 1895. VII. 304.—Ch. C. 1895. II. 1166.—Z. g. Br. 1896. XIX. 191.

WOOD,
I. Enzyme Action in Lower Organisms.—Proceedings of the Royal Society of Edinburgh. 1889. XVII. 27.—C. f. B. 1890. VIII. 266.

WOOD, Joseph T.,
I. Methods of Bacteriological Research, with some Account of Bran Fermentation.—Journal of the Society of Chemical Industry. 1890. January 31 and December 11.
II. Gärung in der Lederindustrie.—Chemical News. 1893. LXVIII. 109.—Ch. C. 1893. II. 1013.—K. J. IV. 256.
III. Fermentation in the Leather Industry. —Journal of the Society of Chemcial Industry. 1894. XIII. 218.—Ch. C. 1894. I. 941.—K. J. V. 271.

WOOD, Joseph T., and WILLCOX, W. H.,
I. Further Contribution on the Nature of Bran Fermentation. — Journal of the Society of Chemical Industry. 1893. XII. 422.—Ch. C. 1893. II. 214.—K. J. IV. 256.

WOOD-SMITH, R. F.,
I. The Bacteriology of Yeast. Part I.—Journal of the Federated Institutes of Brewing. 1898. IV. 115.—W. f. Br. 1898. XV. 161.—Ch. C. 1898. I. 1141. —K. J. IX. 134.

WORONIN, M.,
I. Über die bei der Schwarzerle (Alnus glutinosa) und der gewöhnlichen Gartenlupine (Lupinus mutabilis) auftretenden Wurzelanschwellungen. — Mémoires de l'Acad. des sciences de St. Pétersbourg. 1866. 7ème série. T. 10. No. 6.—Bot. Zeitg. 1866. XXIV. 329.

WORTMANN, Julius,
I. Untersuchungen über das diastatische Ferment der Bakterien.—Z. f. physiolog. Chemie. 1882. VI. 287.—Ch. C. 1882. 593.
II. Mitteilung über die Verwendung von konzentriertem Most für Pilzkulturen. Bot. Ztg. 2. Abt. 1893. LI. 177.—C. f. B. 1893. XIV. 816.
III. Über den sogen. Stopfengeschmack der Weine und seine Bekämpfung. Vortrag.—Ber. ü. d. XV. D. Weinbau-Kongress in Heilbronn. 1896. 44.—Weinbau und Weinhandel. 1896. No. 45.—K. J. VII. 148.
IV. Untersuchungen ü. d. Auftreten u. Verhalten von Dematium pullulans im gärenden Most. — Jahresber. d. kgl. Lehranstalt zu Geisenheim a. Rh. für 1891-92. 52.—K. J. III. 165.
V. Die seitherigen Erfahrungen der Praxis mit reinen Hefen und die Konsequenzen, welche sich hieraus für die Züchtung sowie die Anwendung der Reinhefen ergeben.—Ber. ü. d. Verh. d. XIII. D. Weinbau-Kongresses in Mainz. 1894. 57.—C. f. B. 2. Abt. 1895 I. 249.—Ch. C. 1895. I. 937.—K. J. V. 168.
VI. Einige Beobachtungen über das Verhalten der Hefen im Weinberge.—Bericht der kgl. Lehranstalt, etc., zu Geisenheim a. Rh. pro 1897-98. 75.—Weinbau u. Weinhandel. 1898. 278.—Z. g. Br. 1898. XXI. 715.
VII. Vorkommen und Wirkung lebender Organismen in fertigen Weinen und ihre Bedeutung für die Praxis der Weinbereitung.—Landw. Jahrbücher. 1898. XXVII. 631.—C. f. B. 2. Abt. 1899. V. 229.—Ch. C. 1899. I. 71.
VIII. Über die Herkunft der Weinhefen.—Bericht d. Kgl. Lehranstalt, etc., zu Geisenheim a. Rh. pro 1895-96. 82.—K. J. VII. 35.
IX. Über die vermeintliche Hefebildung von Aspergillus oryzæ.—*Ibid.* 84.—K. J. VII. 36.
X. Über die Ursache des zögernden Eintrittes der Gärung der 1895 er Moste.—Weinbau und Weinhandel. 1895. No. 45.—K. J. VI. 148.
XI. Über künstlich hervorgerufene Nachgärungen von Weinen in der Flasche und im Fasse.—Landw. Jahrbücher. 1897. XXVI. 473.—C. f. B. 2. Abt. 1898. IV. 588.—Ch. C. 1897. II. 506.
XII. Untersuchungen über das Umschlagen der Weine. — Weinbau u. Weinhandel. 1899. S. 294.—C. f. B. 2. Abt. 1900. VI. 298.—Ch. C. 1900. I. 1311.—K. J. X. 145.

XIII. Zur Kenntnis der Reizbewegungen. —Bot. Ztg. 1887. XXXXV. 785.
XIV. Ein Beitrag zur Biologie der Mucorineen.—Bot. Ztg. 1881. XXXIX. 368.
XV. Anwendung und Wirkung reiner Hefen bei der Weinbereitung. Berlin. 1895. Parey. 2*s.* 6*d.*—K. J. VI. 172.
XVI. Landw. Jahrb. 1892. XXI. 901.
XVII. Vorkommen u. Wirkung lebender Organismen infestigen Weinen u. ihre Bedeutung f. d. Weinbereitung. Berlin. 1890.
XVIII. Die wissenschaftl. Grundlagen d. Weinbereitung u. Kellerwirtschaft. Berlin. 1905, p. 45.
XIX. Chem.-Ztg. 1898. XXII. 790.
XX. Landw. Jahrb. 1894. XXIII. 552.
XXI. Bericht d. Kgl. Lehranstalt, etc., zu Geisenheim pro 1900, 1901. 92.

Wossnesenski,
I. Influence de l'oxygène sous pression augmentée sur la culture du Bacillus anthracis.—C. R. 1884. LXXXXVIII. 314.

Wróblewski, A.,
I. Gärung ohne Hefezellen.—Centralblatt f. Physiologie. 1898. XII. 697.—Ber. d. D. Chem. Ges. 1898. XXXI. 3218. —Ch. C. 1899. I. 500.—K. J. IX. 321.
II. Zusammensetzung des Buchner'schen Hefepresssaftes.—Ber. d. D. Chem. Ges. 1898. XXXI. 3218.—C. f. B. 2. Abt. 1899. V. 161.—K. J. X. 320.
III. Über den Buchner'schen Hefepresssaft. —Journal f. prakt. Chemie. 1901. CLXXII. 1.—C. f. B. 2. Abt. 1900. VI. 59.—Ch. C. 1899. II. 672.—K. J. X. 324.
IV. Centralbl. f. Physiologie. 1899. XIII. 284.
V. J. f. pr. Chem. 1901. 2nd Series. LXIV. 1.
IV. [The reference thus made in Chap. 65 should be VI.] Ber. d. D. Chem. Ges. 1898. XXXI. 1134.

Wulff, Carl,
I. Beiträge zur Kenntnis der Nucleinbasen. —Z. f. physiolog. Chemie. 1893. XVII. 468.—Ch. C. 1893. I. 421.

Wurm, Emanuel,
I. Über Essigbildung mittelst Bakterien.—Dingler's Journal. 1880. CCXXXV. 225.

Wurtz,
I. Liebig's Ann. 1855. XCIII. 107.

Wurtz, R., et Foureur, A.,
I. Note sur un procédé facile de culture des microorganismes anaérobies. —Archives de médecine expérimentale et d'anatomie pathologique. 1889, p. 523.—C. f. B. 1889. VI. 710.

Wurtz, R., et Leudet, R.,
I. Note sur l'identité du bacille lactique de Pasteur avec le Bacillus lactis aërogenes. —Comptes rendus de la société de Biologie. 1893. 531.—K. J. IV. 196.

Wüthrich, E., und Freudenreich, É. von,
I. Über den Einfluss der Fütterung auf den Bakteriengehalt des Kuhkotes.—C. f. B. 2. Abt. 1895. I. 873.—Ch. C. 1896. I. 452.

Wyschnegradsky,
I. Liebig's Ann. 1878. CXC. 350.

Wysmann,
See Wijsmann.

Wyss, O.,
I. Über den Milchschlamm und darin sich findende Mikroorganismen.—Tageblatt der 62 Versammlung Deutscher Naturforscher u. Ärzte. 1889. 501.—C. f. B. 1889. VI. 587.

Wyssokowitsch (auch Wyssokowicz),
I. Einfluss des Ozons auf das Wachstum der Bakterien.—Mitteilungen aus Dr. Bremer's Heilanstalt für Lungenkranke in Görbersdorf. 1890. Wiesbaden. Bergmann.—C. f. B. 1890. VIII. 662. —K. J. I. 45.

Yabe, K.,
I. Über einen vegetabilischen Käse aus Sojabohnen. — Landw. Versuchs-Stationen. 1895. XXXXV. 438.—C. f. B. 2. Abt. 1895. I. 413.—Ch. C. 1895. I. 894.—K. J. VI. 293.
II. Preliminary Note on Saké Yeast.— Bulletin of the Imperial University of Tokyo, College of Agriculture. 1896. II. 219.— K. J. VII. 94.
III. On the Origin of Saké Yeast (Saccharomyces saké).—Bulletin, etc. 1897. III. 221.—C. f. B. 2. Abt. 1898. IV. 554.
IV. On Two New Kinds of Red Yeast.— Bulletin, etc. 1897. III. 233.—C. f. B. 2. Abt. 1898. IV. 555.
V. Quoted in Chem. Centralbl. 1894. II. 1048.
VI. Imp. University of Tokyo, College of Agricult. Bull. No. 3, p. 232.—Abst. in Chem. Centralbl. 1897. II. 818.
See also Kosai und Yabe.

Yoshii, T.,
See Kellner und Yoshii.

Yoshimura, K.,
I. Notiz über das Verhalten von Hippursäure im Boden.—College of Agriculture Bulletin. 1895. II. 221.—Ch. C. 1896. I. 56.

Yung, E.,
See Pictet et Yung.

Zacharias, E.,
I. Beiträge zur Kenntnis des Zellkerns und der Sexualzellen. Bot. Ztg. 1887. XXXXV. 281.
II. Über Chromatophilie.—Ber. d. D. Bot. Ges. 1893. XI. 188.—Gives the antecedent bibliography.

ZAHN, F. W.,
I. Untersuchungen im Blute gesunder Tiere.—Virchow's Archiv. LXXXXV. 401.

ZALESKI, St. Szcz. von,
I. Chemische Untersuchungen und Unternehmungen in Sibirien. Mitteilungen über Darstellung von Arakà oder Ojrán, berauschendes Getränk aus Milch.—Chem.-Ztg. 1895. XIX. 77.—K. J. VI. 225.

ZALEWSKI, A.,
I. O tworzeniu sie zarodnikow w komórkach drożdży.—Rozprawy, etc., Akademii Umiejętności w Krakowie. 1886. XIII. 124.—Botan. Centralblatt. 1886. XXV. 1.

ZANDER, Enoch,
I. Vergleichende und kritische Untersuchungen zum Verständnisse der Jodreaktion des Chitins.—Dissert. Erlangen.—Pflüger's Archiv. 1897. LXVI. 545.—Ch. C. 1897. I. 1237.

ZANGEMEISTER, Wilhelm,
I. Kurze Mitteilungen über Bakterien der blauen Milch.—C. f. B. 1. Abt. 1895. XVIII. 321.—Ch. C. 1896. I. 316.

ZEGA, A., und MAJSTOROVIC, R.,
I. Der Mais als Volksnahrung in Serbien.—Chemiker-Ztg. 1899. XXIII. 544.—Ch. C. 1899. II. 317.

ZEIDLER, A.,
I. Beiträge zur Kenntnis einiger in Würze und Bier vorkommender Bakterien.—W. f. Br. 1890. VII. 1213.—C. f. B. 1891. IX. 10.—K. J. I. 72.

ZENTHÖFER,
I. Über das Verhalten von Cholerakulturen in Hühnereiern.—Z. f. Hygiene. 1893. XVI. 362.—C. f. B. 1894. XV. 752.—Ch. C. 1894. I. 872.

ZIMMERMANN,
I. C. f. B. 2. Abt. 1902. VIII. 218.
II. *Ibid.* 1901. VIII. 923.

ZIMMERMANN, Albrecht,
I. Über die Fixierung der Plasmolyse.—Zeitschrift f. wissenschaftl. Mikroskopie. 1892. IX. 181.
II. Die Morphologie und Physiologie des pflanzlichen Zellkernes. Jena. 1896. Fischer. *5s.*
III. Morphologie und Physiologie der Pflanzenzelle. Leipzig. 1887.
IV. Sammel-Referate aus dem Gesamtgebiete der Zellenlehre.—Botan. Centralblatt. 1893. Beihefte. 206, 420.

ZIMMERMANN, O. E. R.,
I. Über die Organismen, welche die Verderbnis der Eier veranlassen.—VI. Bericht d. naturwiss. Ges. zu Chemnitz. 1878.—Gives the antecedent bibliography.
II. Das Genus Mucor. Chemnitz. 1871.

ZIPPEL,
I. Vergiftungsversuche mit Penicillium glaucum.—Zeitschrift f. Veterinärkunde. 1894. VI. 57.—Baumgarten's Jahresbericht. 1894. X. 461.—Deutsche Zeitschrift f. Tiermedizin, etc. 1894. XX. 448.

ZIRN, Georg,
See WEIGMANN und ZIRN.

ZOEBL, A.,
I. Braunspitzige Gerste.—Allgem. Brauer- und Hopfenzeitung. 1892. No. 106.—Bot. Centralbl. 1892. LII. 344.

ZÖRKENDÖRFER, C.,
I. Über die im Hühnerei vorkommenden Bakterienarten nebst Vorschlägen zu rationellen Verfahren der Eikonservierung.—A. f. Hygiene. 1893. XVI. 369.—C. f. B. 1893. XIV. 141.—K. J. IV. 107.

ZOPF, Wilhelm,
I. Die Spaltpilze. 3rd Ed. Breslau. 1885.
II. Zur Kenntnis der Organismen des amerikanischen Baumwollsaatmehls. I. Bacterium vernicosum.—Beiträge zur Physiologie und Morphologie niederer Organismen. Leipzig. 1892. A. Felix.—C. f. B. 1893. XIII. 276.—K. J. III. 91.
III. Über Bacterium merismopedioides.—Ber. d. Botan. Vereins d. Provinz Brandenburg. 1882.
IV. Über Pilzfarbstoffe.—Bot. Ztg. 1889. XXXXVII. 53.—Ch. C. 1889. I. 291.
V. Über Ausscheidung von Fetifarbstoffen (Lipochromen) settens gewisser Spaltpilze.—Ber. d. D. Bot. Ges. 1891. IX. 22.—Ch. C. 1891. I. 673.—K. J. II. 84.
VI. Entwicklungsgeschichtliche Untersuchungen über Crenothrix polyspora, die Ursache der Berliner Wasserkalamität.—Berlin. 1879.
VII. Zur Morphologie der Spaltpflanzen. Leipzig. 1882.
VIII. Über die Ursache der Rotfärbung eines neuen Wasserspaltpilzes aus der Familie der Cladotricheen, Sphærotilus roseus.—Beiträge z. Phys. u. Morph. nied. Org. Heft 2. Leipzig. 1892. A. Felix.—C. f. B. 1893. XIII. 234.—K. J. III. 84.
IX. Zur Kenntnis des regressiven Entwicklungsganges der Beggiatoen nebst einer Kritik der Winogradsky'schen Auffassung betreffs der Morphologie der roten Schwefelbakterien.—Beiträge z. Phys. u. Morph. nied. Org. Heft 5. 37. Leipzig. 1895.—K. J. VI. 29.
X. Die Pilze in morphologischer, biologischer und systematischer Beziehung. Breslau. 1890.—Z. g. Br. 1890. XIII. 399.
XI. Die Conidienfrüchte von Fumago.—Nova acta der Ksl. Leop.-Carol. Deutschen Akad. d. Naturf. 1878. XXXX, No. 7, p. 20.

XII. Kritische Bemerkungen zu Brefeld's Pilzsystem.—Beiträgez. Phys. u. Morph. nied. Org. Heft 3. Leipzig. 1893.—C. f. B. 1. Abt. 1893. XIV. 453.
XIII. Oxalsäuregärung (an Stelle von Alkoholgärung) bei einem typischen (endosporen) Saccharomyceten, S. Hansenii n. sp.—Ber. d. D. Bot. Ges. 1889. VII. 94.—C. f. B. 1. Abt. 1889. V. 796.
XIV. Ber. d. D. Chem. Ges. 1889. VII. 95. — Ber. d. D. Bot. Ges. 1900. XVIII. 32.
XV. Ber. d. D. Chem. Ges. 1889. VII. 94.
See also Liesenberg und Zopf.

Zschokke, A.,
I. Über den Bau der Haut und die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte.—Landw. Jahrbuch der Schweiz. 1897. XI. 153.—V. Jahresber. d. Versuchs-Station zu Wädensweil. 1894–95. S. 56.—C. f. B. 2. Abt. 1898. IV. 839.

Zukal, H.,
I. Öster. Botan. Zeitschr. 1893. No. 5.
II. Sitz. d. K. Akad. Wiss. Wien. Math.-naturw. Kl. 1. Abt. 1887. XCVI. Nov. Heft.
III. *Ibid.* 1889. 1. Abt. XCVIII. 561.

INDEX.

An asterisk affixed to a page number indicates that a figure occurs there.

Abiogenesis, i. 3
Abrin, i. 234
Abrus precatorius, i. 234
Absidia, ii. 54
Acetal, ii. 372
Acetaldehyde, ii. 372, 377, 380
Acetamide, ii. 275
Acetate of ammonium. *See* Ammonium acetate
of potassium. *See* Potassium acetate
of sodium. *See* Sodium acetate
Acetic acid, ii. 150, 262, 265, 276, 348, 363, 365, 371, 387
action of, on yeast, ii. 180
on Saccharomycetes apiculatus, ii. 322
Mycoderma on, ii. 310, 311
on proteolysis, ii. 411
derivative from chitin, ii. 27
formation of, by Bacillus ethaceticus, i. 137
by B. ethacetosuccinicus, i. 137
by B. pneumoniæ, i. 137
by bacillus of symptomatic anthrax, i. 140
by B. amylozyme, i. 147
by B. orthobutylicus, i. 148
by Bacterium lactis aerogenes, i. 172
in lactic-acid-tainted wine, i. 193
by Saccharobacillus pastorianus, i. 195
in sweet ensilage, i. 201
in plumped hides, i. 206
in tanning bark liquors, i. 206
by Actinobacter du lait visqueux, i. 215
by Actinobacter polymorphus, i. 215
during the loss of colour by some bacteria, i. 240, 241
in mannitic fermentation, i. 241
by Clostridium pasteurianum, i. 270
in olive oil, i. 311
by Willia anomala, ii. 214
by Torulaceæ, ii. 297
by Mycoderms, ii. 310, 311
by Mycoderma lebenis, ii. 311
by Saccharomyces apiculatus, ii. 318, 323, 324
Acetic acid bacteria, mutability, i. 28
in natural wine must, i. 67
effect of ethyl alcohol on, i. 66, 67
discovery of, i. 295, 296
morphology of, i. 296–298
behaviour of the mucinous envelopes of, towards iodine solution, i. 297
appearance of colonies on solid media, i. 298
morphological influence of temperature on, i. 298–302
behaviour of, towards electricity, i. 302
towards light, i. 302
fermentation, in the digestive system, i. 151
in lambic, i. 196
Liebig's theory of, i. 295
of Mycoderms, i. 296
equation of, i. 302–305
Acetic ether, ii. 215, 379
produced by Saccharomyces apiculatus, ii. 324
Acetone, ii. 351, 355, 365
Achroocellulose, ii. 108
Achroodextrin, ii. 400
Acidophil, ii. 121
Acids, ii. 387
action of, on micro-organisms, ii. 179
on invertase, ii. 387
on proteolysis, ii. 411
on philothion, ii. 416
produced by yeasts, ii. 171
by Torulaceæ, ii. 297, 302
in alcoholic fermentation, ii. 370
Acrogenous branching, ii. 3, 4
Acropetalous growth, ii. 4, 279
Actinobacter polymorphus, ropy milk, i. 215
Adenin, ii. 119, 120, 412
Adenylic acid, ii. 119
Adipocere, i. 244
Æcidia, ii. 17
Aeration of yeast, ii. 271, 272
Aerobes, definition, i. 139, 140
Aerobic yeast, ii. 90
Æsculin, fission of, ii. 269
Æthalium septicum, occurrence of glycogen in, ii. 123
isolation of paracholesterin from, ii. 127

Afral, action of, on Mycoderma cerevisiæ, ii. 313
Agar-agar, i. 100
peptonised bouillon, i. 100
unhopped wort, i. 100
Agaricus arvensis, manganese in, ii. 35
campestris, membrane in, ii. 25
cell wall, ii. 26
chitin in, ii. 26
respiration in, ii. 42
deliciosus, manganese in, ii. 35
melleus, heliotropism in, ii. 39
sanguineus, blueing of, i. 311
Age of sowing of yeast, influence of, on reproductive capacity and power, ii. 168
Air, analysis of germs in, ii. 160
decomposition by, i. 20
influence of, on the fatty constituents of butter and cheese, i. 152, 153
supply of, for ascospores, ii. 94
in films, ii. 307
Albumen, ii. 345
action of Aspergillus niger on, ii. 274, 275
digestion of, by endotryptase, ii. 411
from decomposition of paranucleins, ii. 118
liquefaction of, by yeast, ii. 409
Albuminoid mucinous substances, ii. 132–135
Albumoses, action of endotryptase on, ii. 412, 413
Albuminoids, loss of digestible, by brown hay, i. 200; by sweet ensilage, i. 202; by sour fodder, i. 202
products of putrefied, i. 224–226
poisons, i. 234, 235
protective, ii. 235
Alcohol, ii. 276, 345, 348, 362, 363, 364, 365, 366, 367, 369, 371–375
action of, on reproductive yeast cells, ii. 175; on fermentation, 177, 350; on Mycoderma, 313; on enzymes, 351; on invertase, 387; on maltase, 389; on melibiase, 392; on proteolysis, 411; on yeast, 415
behaviour of yeast gum towards, ii. 128; of yeast cells towards, ii. 175–178
decomposed by Willia anomala, ii. 214; by Mycoderma, 311
decomposition of, ii. 273
early history of, ii. 359, 360
effect of, on Pichia californica, ii. 212; on Pichia taurica, 213; on enzymes, 261, 269
fermentation, ii. 359; in natural wine must, i. 66; and intermolecular respiration, ii. 57, 58, 59, 62; by Penicillium glaucum, 58; by Aspergillus niger, 58; yield by species of Mucor, 59; heat liberated during, 348
influence of, on the growth of races of Saccharomyces apiculatus, ii. 319, 320
production of, ii. 272, 273; by Mucor Rouxii, 65; by Mucor javanicus, 68; by asporogenic yeast cells, 194; by Carlsberg bottom yeast No. 1, 196; by Saccharomyces cerevisiæ, 196; by Saccharomyces Marxianus, 207; by Saccharomyces exiguus, 207; by Saccharomyces fragilis, 209; by Saccharomycodes, 210; by Willia anomala I., 214; by Willia anomala II., 214; and III., 215; by Schizosaccharomyces octosporus, 217; by Schizosaccharomyces mellaceus, 217; by Allescheria Gayoni, 257; by Aspergillaceæ, 272, 273; by Saccharomyces Kefyr, 293; by Torulaceæ, 296, 301; by Saccharomyces apiculatus var. parasiticus, 317, 318; by S. a. var. ellipsoideus, 319; by Saccharomyces apiculatus, 321; by S. apiculatus from beer wort, 321; and from grape and other juices, 321, 322; by Monilia candida, 331; by Monilia javanica, 333; by Monilia albicans, 334; by Oidium lactis, 337; by yeasts, 341, 342; during fermentation, 359–365
Alcohol, source of citric acid, ii. 267
Alcohols, effect of, on reproduction of Torulaceæ, ii. 295
Alcoholase, discovery of, and attempts to isolate it, ii. 340–342; not extracted from yeast by dialysis, filtration, or centrifugalisation, 347–348; properties of, 352–358, 367; endotryptase antagonistic to, 415
Aldehydes, ii. 365, 378, 403
luminous in alkaline solution, i. 125
occurrence in cultures of Mucor racemosus, ii. 60
action of, on proteolysis, ii. 411
Aldehydesulphurous acid, i. 85
Alditriose, ii. 381
Alexine, i. 235
Algæ, i. 22, ii. 3; pure cultures of, i. 103; aluminium as a nutriment for, ii. 32, 36; calcium as food for, 33, 34; arsenic as a substitute for phosphorus, 37
Algic fungi, ii. 3
Alkalies, ii. 31, 387; influence of, on alcoholic fermentation, ii. 348, 349; action of, on proteolysis, 411; on philothion, 416
Alkalinity of nutrient media, influence of, on the development of bacteria, i. 95
Alkanna tincture, ii. 86
Allantoin, i. 253, ii. 275
Allescheria, ii. 257, 276
action on gum arabic, ii. 270; on fats, 271; on acids and alcohols, 276
description and properties of, ii. 219, 221
Gayoni, ii. 257, 258, 261, 269, 270, 395, 397; fission of glucosides by, ii. 269; alcohol produced by, 272, 365; action of, on sugars, 272
Alloxuric bases produced by yeast, ii. 412
Alnus, nodules of, i. 263
Alpinia galanga, ii. 67

Alternaria tenuis, fixation of free nitrogen by, i. 270
Aluminium, ii. 36
chloride, ii. 405; action of, on yeasts, ii. 179
sulphate, ii. 349; action of, on yeasts, ii. 179
Amanita, oxydase in, i. 311
muscaria, muscarine in, i. 233; manganese as food for, ii. 35; casein-dissolving enzyme in, 46; glycogen-formation by, 124
Amblyosporium, description, ii. 219
Amide, influence of, on frothing fermentation, ii. 134, 135
Amides as food for yeasts, ii. 154, 155
Amines, ii. 380
Amino acids, ii. 274, 349, 409, 412, 414
Amitosis, ii. 110
Amitotic division of the nucleus, i. 44
Ammonia, ii. 270, 274, 275, 380, 412, 413; for preserving milk, i. 161; development of, from soil albuminoids, i. 235, 237; as food for yeasts, ii. 154; occurrence in Emmenthal cheese, i. 243; from fermentation of urea, i. 254; production of, by Penicillium brevicaule, ii. 253; effect of, on the reproduction of Torulaceæ, ii. 295
Ammonia salts, ii. 323, 324; a source of nitrogen for Chlamydomucor oryzæ, ii. 68
Ammonium, motion inhibited by, i. 32
acetate, ii. 150
benzoate, ii. 150
bicarbonate, ii. 371
chloride, ii. 349, 405; influence on the conidia-formation by Aspergillus niger, ii. 17
citrate, ii. 150
dextro-tartrate, ii. 150
fluoride, ii. 349
formate, ii. 150
gallate, ii. 150
lactate, ii. 150
malate, ii. 150
nitrate, ii. 311, 405; influence on conidia-formation, ii. 17
oxalate, ii. 128, 150
phosphate, ii. 155, 311; in preparation of mead, ii. 145; influence of, on Schizosaccharomyces Pombe, ii. 191
salicylate, ii. 150
succinate, ii. 150
sulphate, ii. 349, 351, 405; as a nutrient medium, ii. 154; influence on the formation of conidia by Aspergillus niger, ii. 17
tartrate, ii. 152, 311; on the preparation of mead, ii. 145; as a nutrient medium, i. 94
Amœbæ, pure culture, i. 103; yeast similar to, ii. 87
Amœbobacter, i. 282
Amorphophallus Konjaku, ii. 382
Amygdalic acid, ii. 269
Amygdalin, ii. 45, 150, 391; fission of, 269, 270
Amyl alcohol, ii. 276, 376, 377, 378
Amylase, ii. 270, 399–403; secreted by Aspergillus oryzæ, 260, 261; and Asp. niger, 261
Amylo process, ii. 69–71, 377
Amylobacter, meaning of term, i. 144
Amylocarpus, description, ii. 219
Amylodextrin, ii. 68, 400
Amyloins, ii. 399, 400
Amylomaltase, ii. 261
Amylomyces, α-, ii. 65, 397
β-, ii. 65, 66,* 403
γ-, ii. 65, 66,* 397, 403
Rouxii. *See* Mucor amylomyces Rouxii
Anacardiaceæ, i. 309
Anaerobes, definition, i. 139, 140
culture of, i. 140
Pasteur's method, i. 140, 141
puncture method, i. 141
Max Gruber's method, i. 141*
Buchner's pyragallol tube, i. 141,* 142
Fränkel's anaerobic tube, i. 142*
in mixed culture, i. 143
plate cultures, i. 143
Anaerobiosis, i. 139
Anaerobism, ii. 374, 375
Analysis, biological, of brewing water, i. 98, 99
quantitative bacteriological, of water, i. 102
Ang Khak, ii. 11
Anguillula aceti, i. 307
Aniline violet, ii. 107
Animalcula (Monadina), i. 12, 69
Anisotropism of cell wall, i. 30
Anixiopsis stercoraria, ascospores, ii. 21
Antagonism, i. 67
Anthrax, preventive inoculation for, i. 60
Antiformin, action on Mycoderma cerevisiæ, ii. 313
Antigermin, action on Mycoderma cerevisiæ, ii. 313
Antinonnin as an antiseptic, i. 88, 89, 293
Antipeptone, ii. 349
Antiseptics, mineral, i. 86; efficiency of, 86; organic, 87; influence of, on enzymes, 230, 231
Aphanoascus, description, ii. 218
Apothecium, ii. 72
Appert's preservative process, i. 11, 161, 168
Apple enzyme, i. 310; brown spotting by, 310
must, ii. 158
Appressorium, ii. 45, 55
Arabin, ii. 129
Arabinose, ii. 150, 151, 382; behaviour with Mucor Rouxii, ii. 65; fermented by Bac. ethaceticus, i. 137; glycogen-formation by, ii. 125; non-ferment of, ii. 215
Arachis hypogæa, ii. 333; nodule-formation by, i. 263
Arbutin, fission of, ii. 269
Arginase, ii. 412

Arginin, ii. 274, 412
Armillaria mellens, ii. 74
Aromatic bodies produced by yeast, ii. 405
Arrack, ii. 67
Arrowroot, ii. 68
Arsenic, ii. 11, 37, 275; test for, 252 mirror, ii. 38
Arsenical colours, ii. 38
Arsenious acid, ii. 37, 38
Arsenite of potash. *See* Potassium arsenite of soda. *See* Sodium arsenite
Arsenites, ii. 348, 349, 387
Arseniuretted hydrogen, ii. 37, 275
Arthrospores, i. 51, 72; ii. 18
Arthrosporic bacteria, i. 28
Artichoke, i. 186
Asci, ii. 219 *et seq.*, 250, 255
Ascococcus mesenteroides, i. 208
Ascogone, ii. 72
Ascogonnin, ii. 247
Ascoidea, spores, ii. 79 rubescens, spores, ii. 75
Ascomycetes, endogenous spores, ii. 17, 72; glycogen in, 123; sporangia in, 12
Ascospores, ii. 12, 78, 241, 244, 245, 246, 247, 250, 253, 281, 283, 314, 315; of Endomyces decipiens, 74*; of Sacch. anomalus, 74; of yeast cells, 94; conditions for inception of, 96; formation of, 97*; structure of, 99; resisting powers, 102, 103*; karyokinesis of, 109; refractive power, 111
Ascus, ii. 12, 72
Asparagin, ii. 150, 154, 156, 166, 167, 369; chemotaxis, i. 41; influence of Bacillus subtilis on, 133, 134; chemotropism, ii. 43; nitrogen source for Chlamydomucor oryzæ, 68; a glycogen-former in yeasts, 125; as nutriment for Torulaceæ, 293
Aspartic acid, ii. 150, 369, 412
Aspergillaceæ (Mucedineæ), ii. 219, 250; morphology, 218 *et seq.*; affinities, 218; genera of, 218, 219; chemical activity of, 259–277; pathogeny, 260; acids produced by, 261–267
Aspergillin, ii. 238, 275
Aspergillus, ii. 403; action on oxalates, ii. 276; affinities, ii. 242, 243, 288; decomposition effects, ii. 264; description, ii. 219, 220, 221; influence of cadmium on, ii. 33; in preparation of nutrient yeast, ii. 124; pathogenic species, ii. 225, 232, 233, 234, 235, 238, 239, 240, 241
Aspergillus albus, sterigmata, ii. 227, 240
atropurpureus, ii. 240; sterigmata, ii. 227
auricomus, ii. 242; sterigmata, ii. 227
bronchialis, ii. 234
cæsiellus, ii. 227
calyptratus, ii. 236; sterigmata, ii. 227
candidus, descriptive, ii. 222,* 223, 240; conidia and conidiophore, ii. 226; influence of temperature on, ii. 227; liquefaction of gelatin by, ii. 46, 273 membrane lignification, ii. 30
Aspergillus citrisporus, ii. 236; sterigmata, ii. 227
clavatus, ii. 222, 223,* 225*; affinity, ii. 241; conidiophore and conidia, ii. 226; descriptive, ii. 235, 236; influence of temperature on, ii. 227; liquefaction of gelatin by, ii. 273; sterigmata, ii. 227
conoideus, ii. 30
Delacroixii, ii. 236
elegans, ii. 242; sterigmata, ii. 227
flavescens, ii. 233, 242, 259; conidia-formation, ii. 21; lignin, ii. 30; respiration, ii. 42
flavus, ii. 252; affinity, 228; arsenic, ii. 38; conidia, ii. 22, 226; conidiophore, 226; descriptive, ii. 220, 223, 224, 225, 232, 233,* 242, 259; fission of cotton-seed oil by, ii. 271; influence of temperature on, ii. 227, 232; lipase secreted by, ii. 271; liquefaction of gelatin by, ii. 46, 273; malformations, ii. 226; pathogeny, ii. 233, 275; sterigmata, ii. 227
fumigatus, ii. 242, 246, 259, 260; action on milk, casein, albumen, fibrin, ii. 274, 275; affinities, ii. 235, 240; conidia, ii. 22, 226, 232; conidiophores, ii. 226; decomposing action, ii. 269; descriptive, ii. 220, 222,* 223,* 233,* 234; fermentation of tobacco by, i. 129; fission of glucosides by, ii. 269; heating of barley by, i. 127; influence of temperature on, ii. 227; inulase secreted by, ii. 270; lipase secreted by, ii. 271; liquefaction of gelatin by, ii. 46, 273; malformations, ii. 226; pathogeny, ii. 234, 275, 276; saccharification by, ii. 261; sterigmata, ii. 227
fuscus, ii. 270
giganteo - sulfureus, sterigmata, ii. 227
giganteus, ii. 222*; conidia and conidiophore, ii. 226, 236, 237; liquefaction of gelatin by, ii. 273; sterigmata, ii. 226, 227, 237
glaucus, action on milk, casein, albumen, fibrin, ii. 274, 275; on arsenical substances, ii. 275; on starch, ii. 261; affinities, ii. 235, 241; alcohol produced by, ii. 272; behaviour with arsenic, ii. 37; cellulose in, ii. 25; chitin, local distribution in cell wall, ii. 28; colouring-matter produced by, ii. 275; conidia, ii. 21, 226; decomposition effects of, ii. 269; descriptive, ii. 220, 222,* 223,* 224,* 231*; fission of cotton-seed oil by, ii. 271; fission of glucosides by, ii. 269; influence of light on, ii. 40, 41, 42; influence of temperature on, ii. 227; inulase secreted by, ii. 270; lignification,

ii. 30; lipase secreted by, ii. 271; liquefaction of gelatin by, ii. 46, 273; malformations, ii. 226; occurrence, ii. 240; oxalic acid produced by, ii. 142; pathogeny, ii. 225; products formed from oxalic acid, ii. 142; saccharification by, ii. 261; sterigmata, ii. 227
Aspergillus griseus, ii. 242, 259
herbariorum, ii. 230
Koningi, ii. 236
Lignieresi, ii. 234
luchuensis, ii. 225; descriptive, ii. 234, 235; sterigmata, ii. 227
luteus, ii. 242; conidia-formation, ii. 41
medius, ii. 225, 275
minimus, ii. 223, 236; conidia and conidiophores, ii. 226; liquefaction of gelatin by, ii. 273; sterigmata, ii. 227
nidulans, affinity, ii. 241; ascospores, ii. 234; conidia and conidiophore, ii. 226; descriptive, ii. 220, 222,* 223, 224, 225, 240, 241*; influence of temperature on, ii. 227; lipase secreted by, ii. 271; pathogeny, ii. 275, 276; sterigmata, ii. 227
niger, ii. 252, 262, 395, 396, 399; action of, ii. 269; action on acids, ii. 276; action on fats, ii. 271; action on fibrin, gelatin, and albumen, ii. 274; action on milk, casein, albumen, fibrin, ii. 274, 275; action on peptone, ii. 274; on starch, ii. 261; affinities, ii. 242; alcohol produced by, ii. 272; alcohol a nutrient material for, ii. 272; amidases produced by, ii. 275; behaviour towards aluminium, ii. 36; behaviour towards chloride of potassium, ii. 32; behaviour towards iron and the allied metals, nickel, cobalt, and manganese, ii. 34–36; cæsium as nutrition for, ii. 31; chemical analysis, ii. 22; chemotropism, ii. 43, 44; chitin in, ii. 28; colouring-matter produced by, ii. 275; conidia and conidiophore, ii. 16, 17, 22, 32, 226; decomposing effects of, ii. 270; decomposition of sugars by, ii. 269; description, ii. 220, 223,* 225, 237, 238,* 239; diastase-formation, ii. 47; effect of lack of free oxygen, ii. 58; enzymes secreted by, ii. 268; influence of temperature on, ii. 227; inverting action of, ii. 260; fat-decomposing enzyme, ii. 47; fission of glucosides by, ii. 270, 271; fission of polysaccharides by, ii. 270; fongose, ii. 29; gelatin-liquefaction by, ii. 46, 273; inulase secreted by, ii. 270; lipase secreted by, ii. 271; malformation, ii. 226; nutritive value of alkalies, ii. 32; oxalic acid produced by, ii. 142, 263, 264, 276; oxydase secreted by, ii. 277; pathogeny, ii. 238; pectinase secreted by, ii. 270; platinum innocuous to, ii. 32; relation of, to arsenic, ii. 37, 38; respiration quotient, ii. 57; saccharification by, ii. 261; sterigmata, ii. 227, 240; tannase secreted by, ii. 271
Aspergillus nigrescens, ii. 234, 237, 242
nigricans, ii. 237, 242
novus, liquefaction of gelatin by, ii. 273
ochraceus, ii. 220, 242; conidiophore, ii. 226
oryzæ, ii. 242, 262; action on cell walls, ii. 270; on milk, casein, albumen, fibrin, ii. 274, 275; on saccharose, ii. 272; on starch, ii. 270; affinity, ii. 232; alcohol produced by, ii. 272; conidia, ii. 22, 226; conidiophore, ii. 226; catalase secreted by, ii. 277; cytase secreted by, ii. 270; decomposing effect of, ii. 269; description, ii. 220, 223,* 225, 227, 228*; diastatic enzyme, ii. 46, 65, 260; fission of glucosides by, ii. 269, 270; influence of temperature on, ii. 227; inulase secreted by, ii. 270; liquefaction of gelatin, ii. 46, 273; malformations, ii. 226, 228; oxydase secreted by, ii. 277; polysaccharides not split up by, ii. 270; saccharification by, ii. 261; Saccharomyces cells in, ii. 80; sterigmata, ii. 227; used in the amylo process, ii. 71
ostianus, ii. 222,* 223; colouring-matter produced by, ii. 275; liquefaction by, ii. 46, 273; sterigmata, ii. 227
penicillopsis, ii. 227, 236
perniciosus, sterigmata, ii. 227
phœnicis, ii. 225; descriptive, ii. 239; sterigmata, ii. 227
pseudoclavatus, ii. 223, 225; affinity, ii. 236; descriptive, ii. 241, 242; sterigmata, ii. 227
pseudonidulans, ii. 241
pulverulenta, sterigmata, ii. 227
quininæ, ii. 242
Rehmii, ii. 223, 224,* 225; conidiophore, ii. 226; descriptive, ii. 242; sterigmata, ii. 227
repens, conidia, ii. 16; conidiophore, ii. 13
spurius, conidiophore, ii. 226; sterigmata, ii. 227
strychni, ii. 227; descriptive, ii. 239
subfuscus, ii. 242; arsenic-reduction by, ii. 38
sulfureus, ii. 222,* 242; sterigmata, ii. 227
syncephalis, ii. 242
terricola, ii. 242; ammonia produced by, i. 235

Aspergillus Tokelau, ii. 223*; conidia, ii. 226; descriptive, ii. 235*; pathogeny, ii. 235; sterigmata, ii. 227
ustilago, ii. 240
variabilis, ii. 242; sterigmata, ii. 227
varians, ii. 222,* 223*; behaviour towards gelatin, ii. 46
versicolor, ii. 227, 242; colouring-matter of, ii. 274; lipase secreted by, ii. 271
violaceofuscus, ii. 240; sterigmata, ii. 227
virens, behaviour towards arsenic, ii. 37
Welwitschiæ, ii. 239, 240
Wentii, ii. 222,* 223,* 225; action on albumen, casein, fibrin, milk, ii. 274, 275; on cells, ii. 270; affinity, ii. 234, 235; conidia, ii. 22, 226; conidiophore, ii. 226; decomposing action of, ii. 269; descriptive, 229, 230*; fission of glucosides by, ii. 269, 270; influence of temperature on, ii. 227; inulase secreted by, ii. 270; liquefaction of gelatin by, ii. 273; saccharification by, ii. 261
Asporogenation, ii. 191–198
Assimilation of carbon dioxide, i. 23; in the dark, i. 114, 291
Attenuation effect of phosphoric acid, ii. 143, 145; of potash, ii. 139
Aurantiaceæ, ii. 262
Autoclave, i. 82
Autodigestion of yeast, ii. 404, 408, 409, 410, 411, 412, 413, 414, 415
Autofermentation, ii. 346, 351, 352; of yeast, ii. 404–407
Autolysis, ii. 408
Auxanogram, i. 104
Auxanography, i. 104
Awamori, ii. 175, 176, 234, 235

Bacille amylozyme, chemical activity, i. 147
Bacillus (long rods), i. 25, 26,* 27, 70, 72, 73
Bacillus acidi lactici, Hueppe, i. 171, 172
acidi lactis, Marpmann, lactic acid formation, i. 172
acidi lævolactici, formation of levolactic acid, i. 178
acidificans longissimus, i. 190
albus, sensitiveness to vibration, i. 64
Bacillus alvei, form of sporing cell, i. 47
amylobacter, i. 145, 149, 150, 152
anthracis, action of carbolic acid on spores, i. 87; of chlorine water on spores, i. 86; of ethyl alcohol on spores, i. 89; of formalin on spores, i. 89, 90; of mercuric chloride on spores, i. 84; culture of, i. 55, 67; diastase-secretion, i. 148; effect of lithium chloride on, i. 36; influence of high pressure, i. 65; influence of light, i. 62; involution forms, i. 29*; occurrence in milk, i. 123; pleochroism, i. 31; pleomorphism, i. 72; resisting power of spores, i. 80, 156, 157; spore-germination, i. 52*
Bacillus arborescens, ammonia-production, i. 235
aromaticus, cheese aroma, i. 247
aurantiacus, orange-red pigment, i. 110
berolinensis indicus, blue pigment, i. 121
butylicus. *See* Granulobacter saccharobutyricum
butyri fluorescens, i. 122
butyricus (Botkin), i. 146
butyricus (Hueppe), i. 143, 144
cœruleus, pigment, i. 121
corallinus, pigment, i. 107
corticalis, behaviour to light, i. 206; chemical activity, i. 206, 207; morphology, i. 206
cyaneo-fluorescens, blue coloration of milk by, i. 116
cyaneo-fuscus, black glue, condition of formation, i. 119; blue coloration of cheese by, i. 118; morphology, i. 118; pigment granules (micro-chemical analysis), i. 118
denitrificans, morphology, i. 236, 237
denitrificans α and β, i. 236
denitrificans II., chemical activity, i. 236
diatrypeticus casei, capsule-formation, i. 31, Plate I.*; chemical activity, i. 249; morphology, i. 249
enteritidis, meat poison, i. 233
erythrosporus, i. 107
ethaceticus, morphology, i. 137; splitting of optically inactive glyceric acid by, i. 178
ethacetosuccinicus, chemical activity i. 137
Fitzianus, conversion of glycerin to ethyl alcohol, i. 136; diastase formed by, i. 148; morphology, i. 136*
fluorescens albus, fluorescence, i. 122
fluorescens liquefaciens, ammonia-production, i. 213; gelatin-liquefier, i. 122; green fluorescent pigment, i. 122; invertin-production, i. 213
fluorescens non-liquefaciens, chemical activity, i. 153; green fluorescent pigment, i. 122
fluorescens putidus, ammonia-producer, i. 235; fluorescence, i. 122
fluorescens tenuis, fluorescence, i. 122
fœtidus lactis, i. 182
granulatus roseus, pigment, i. 107
Guillebeau, blowing of cheese, i. 215, 249; inflammation of udder, i. 215; ropy milk, i. 215
gummosus, i. 218
ilidzensis capsulatus, influence of temperature, i. 60
indicus, pigment, i. 107
indigogenus, fermentation, i. 120
inflatus, form of sporing cell, i. 47*; form and size of spores, i. 49
janthinus, pigment, i. 121
janthinus (Zopf), a mmonia-producer, i. 235

Bacillus lactis acidi (Leichman), formation of levolactic acid, i. 179
lactis acidi (Marpmann), i. 172
lactis acidi cyanogenus, action of light on i. 62; pigment, i. 116; sensitiveness to acids, i. 116; varieties, i. 116
lactis acidi erythrogenes, gelatin-liquefier, pigment, coagulation of milk, i. 108
lactis acidi saponacei, soapy milk, i. 217
lactis acidi viscosus, morphology, i. 215
lactis peptonans (α, β, γ, δ, ϵ), occurrence in milk, i. 158
liodermos (gum bacillus), bread disease, i. 136; occurrence in milk, i. 145
liquefaciens lactis amari, gelatin-liquefier, i. 252; morphology, i. 252
liquefaciens magnus, formation of β-methyl-indol-acetic acid, i. 224; of mercaptan, i. 226
lividus, blue pigment, i. 121
lupuliperda, form, gelatin-liquefier, i. 128; produces butyric acid, i. 128; as also trimethylamine, i. 128
megaterium, ii. 403; diastase, i. 148, ii. 46, 47; effect of vibration, i. 64; influence of lithium chloride, i. 36; morphology, i. 27*; plasmolysis, i. 32; proteolytic enzyme, i. 230; resistance of endospores, i. 50; spore-formation, i. 46*; spore-germination, i. 54*
membranaceus amethystinus, violet pigment, i. 121
mesentericus fuscus, culture on potato and agar, i. 135; peptonising enzyme, i. 135
mesentericus ruber, ammonia-producer, i. 235; morphology, i. 135; resistance of endospores, i. 135
mesentericus vulgatus, ammonia-producer, i. 235; disease in bread, i. 135; enzyme-former, i. 135; occurrence in milk, spores, i. 135, 158; viscous milk, i. 215
mycoides, chemical activity, i. 235
No. 41, butter aroma, i. 181; morphology, i. 181
œdematis maligni, decomposition of carbohydrates, i. 140
oogenes fluorescens α, gelatin-liquefier, pale green pigment-former, putrefaction of eggs, i. 166, 167
oogenes fluorescens, α, β, γ, δ, ϵ, pigment-formation, i. 166, 167; putrefaction of eggs, i. 166
oogenes fluorescens hydrosulfureus, α, β, γ, δ, ϵ, gelatin-liquefier, i. 166
oogenes fluorescens hydrosulfureus, η, θ, ι, putrefaction of eggs, i. 167
orthobutylicus, chemical activity, i. 148
oxalaticus, structure of, i. 34
panificans, disease of bread, i. 135
Bacillus phosphorescens. *See* Photobacterium indicum
pituitosi, ropy milk, i. 215
pneumoniæ crouposæ, capsule-formation, i. 31*; fermentation of indigo, i. 120; producer of ethyl alcohol and acetic acid, i. 137
prodigiosus. *See* Micrococcus prodigiosus
pseudanthracis, influence of light on spore-formation, i. 49
pyocyaneus, a fat-splitter, i. 153; fluorescence, i. 121; influence of electricity, ii. 58; influence of light, i. 62; pigment, i. 121, 122; production of varieties, i. 121; proteolytic enzyme, i. 230
radicicola, culture, i. 264, 265; enriching nutrient solutions in nitrogen, i. 268; morphology, i. 264; penetration of epidermal cells of root-hairs, i. 267
ramosus, colonies on agar, i. 103; denitrification, i. 236; diastase-formation, i. 148; formation of sulphuretted hydrogen, i. 225
rubellus, pigment, endospores, Clostridium, i. 107
ruber, influence of light, i. 62; of vibration, i. 65; pigment, i. 107
saccharobutyricus, i. 147
saprogenes I., II., III., i. 226
saprogenes vini I., morphology, i. 239
saprogenes vini II.–VII., decoloration of wine, i. 239; peptonising enzyme, i. 239
saprogenes vini III., spore-formation, i. 48
Schafferi, blowing of cheese, i. 249; Nissler cheese, i. 249
sessilis, spore-germination, i. 55*
suaveolens, conversion of starch into dextrin and glucose, i. 147
subtilis, ammonia-production, i. 235; behaviour of endospores towards dyes, i. 50; decomposition of sugar, i. 134; dependence of motility upon mode of nutrition, i. 132, 133; diastase-secretion, i. 148; effect of constant minute vibration, i. 64; form of spores, i. 49; germinating period, i. 54; influence of nutritive conditions, i. 29,* 36, 133, 134; morphology, i. 131,* 132*; peptonising enzyme, i. 103, 134, 230; period of generation, i. 44; pure culture, i. 55, 132; resisting power of endospores, i. 50, 80, 81, 131; sulphuretted hydrogen, i. 225
of swine erysipelas, sensitiveness to salt solutions, i. 165; sulphuretted hydrogen produced by, i. 225
tetani, form of sporing cells, i. 47; diastase-formation, i. 148
tetragenus, diastase-producer, i. 148; forms sulphuretted hydrogen, i. 225
thermophilus, influence of temperature, i. 59; nutrient solution with caragheen, i. 101

Bacillus tuberculosis, influence of light on, i. 62; mutability, i. 72; pleochroism, i. 31
tumescens, longitudinal division, i. 42; spore-formation, i. 48*
typhi abdominalis, absorption of phloxin red, i. 34; differentiation from Bact. coli commune, i. 39, 90, 179, 186, 228, 241; influence of formalin, i. 90; of high pressure, i. 65; of hydrogen peroxide, i. 86; of light, i. 61, 62*; of milk of lime, i. 87; mortal temperature, i. 156; occurrence in milk, i. 156; penetration of eggs, i. 167; production of levolactic acid, i. 179; splitting of fat, i. 153; symbiosis with Bac. denitrificans, i. 236
violaceus, gelatin-liquefier, i. 121; influence of light, i. 62
virens, green pigment, i. 122
viridans, fluorescence, i. 122; green pigment, i. 122
viscosus sacchari, morphology, i. 212; mucus-formation, i. 212
viscosus vini, morphology, i. 217
viscosus I. and II., i. 219, 220
viscosus III., influence of food supplied, i. 220; morphology, i. 220
Bacteria, i. 25, 69; ash constituents required, i. 36; chromogenic, i. 105; chromoparous, i. 105, 107; chromophorous, i. 105, 114; coloured and colouring, i. 105; content of, in soil, i. 137; definition, i. 25; heat-resistance, i. 131, 132; iron, i. 272–277; mobility, i. 37; nitrifying, i. 288; nitrogen constituents, i. 35; overgrowth, i. 269; parachromophorous, i. 105; photogenic, i. 123; photogram, i. 62,* 63; pigments, i. 105, 108–110; purple, i. 111–114; putrefactive, i. 226; staining, i. 39; sulphur, i. 278–287; systems, i. 69–74; vegetable nature of, i. 69. *See also* Acetic acid bacteria, Lactic acid bacteria
Bacteridium, i. 39
Bacteriopurpurin, i. 112, 113, 281, 282
Bacterio-spectrogram, i. 113
Bacterium, i. 69, 70
Bacterium aceti, behaviour of cell plasma towards iodine solution, i. 297; branching, ii. 1; chemical activity, i. 295, 296; development temperature, i. 298; form of colonies, i. 103; long thread, i. 299,* 300,* 301,* 303*; morphology, i. 298–304*; mutability, i. 71. Synonymous with Ulvina aceti
chlorinum, behaviour to light, i. 122
chrysogloia, pigment, i. 108, 110
coli commune, ammonia-producer, i. 235; as a lactic acid bacterium, i. 173, 179; denitrification, i. 236, 237; differentiation from Bac. typhi abdominalis, i. 39, 90, 179, 186, 228, 241; formation of neurine, i. 233; formation of nitrites in the intestine, i. 236, 237; gas-formation, i. 228; influence of light, i. 61; influence of lithium chloride, i. 36; lab-production, i. 186; mixed cultures with Bac. typhi abdominalis, i. 67, 236; morphology, i. 228; occurrence in cow-dung, i. 155; toxic action of phenol, i. 87; varieties, i. 228
Bacterium egregium, yellow pigment, i. 108, 110
erythrosporus, fluorescence, i. 122; pigment, i. 107, 122
furfuris, chemical activity, i. 205; morphology, i. 205
gelatinosum betæ, influence of medium, i. 213; inversion, i. 213; motility, i. 213
gliscrogenum, i. 219
gummosum, i. 218
Hessii, formation of ropy milk, i. 216; morphology, i. 216
Kützingianum, behaviour of iodine solution to mucinous envelope, i. 297; development temperature, i. 298; long threads, i. 302; morphology, i. 296, 297*
lactis (Lister), mutability, i. 70, 71; pure culture, i. 97, 171
lactis acidi (Marpmann), lactic fermentation, i. 172
lactis aerogenes, chemical activity, i. 172, 173
limbatum lactis acidi, lactic fermentation, i. 172
lucens, i. 123
Ludwigi, influence of temperature, i. 59, 60
merismopedioides, mutability, i. 71
Pasteurianum, i. 31, ii. 358; behaviour of mucinous envelope to iodine solution, i. 297; change and disintegration of long threads, i. 293, 299,* 300,* 301*; change from short to long threads, i. 298; development temperature, i. 298; long threads, i. 299*; morphology, i. 296, 297; mucin-formation, i. 31; mutability, i. 71
pediculatum, lateral development of mucin, i. 212*
peptofaciens, solution of casein, i. 231
phosphorescens, influence of temperature, i. 59
phosphorescens, F. *See* Photobacterium Fischeri
photometricum, susceptibility to spectrum colours, i. 63
(Bacteridium) prodigiosum. *See* Micrococcus prodigiosus
radicicola. *See* Bacillus radicicola
rubescens, i. 78, 111, 281
sulfuratum, i. 281
syncyaneum (Vibrio cyanogenus), blueing of milk, i. 115; fluorescence, i. 122
synxanthum (Bac. synxanthus), yellow coloration of milk, i. 109
termo, behaviour to albumen-free media, i. 96; meaning of term, i. 226, 227*; tuft of flagella, i. 39
ureæ (Leube), morphology, i. 255

Bacterium ureæ (van Tieghem). *See* Micrococcus ureæ
vermiforme, morphology, i. 197, 198, 212; symbiosis with Sacch. pyriformis, i. 66, 196,* 197*
vernicosum, arthrospores, i. 51
viride, green pigment, i. 122
xylinum, ii. 381; analysis of cell wall, i. 30; behaviour of mucinous envelope, i. 298; chemical activity, i. 305
Zopfii, arthrospores, i. 51; influence of gravity on rate of growth, i. 65; involution forms, i. 29*; mutability, i. 71, 72*. *See* Proteus Zenkeri
Bacteroids, i. 264, 266–269
Barégine, i. 281
Barium, ii. 32
chloride, ii. 349
Bark liquor, bacteria of, i. 206; souring of, i. 205, 206; sugar of, i. 206
Barley, ii. 385, 388; heating of, by Aspergillus fumigatus, i. 127; pentosans in, ii. 151
Basidia, ii. 17
Basidiomycetes, ii. 17; conidiophores, ii. 80; glycogen-formation, ii. 123
Basifugal development, ii. 4, 16, 279
Basipetal development, ii. 15
Béchamp, microzyme theory, i. 7
Beer, ii. 367, 369, 377, 379; action of Torulaceæ on, ii. 298; as food for Torula, ii. 293, 294; for Torulaceæ, ii. 295; bitterness, ii. 98, 103; break in, ii. 135, 136; bright, i. 78, 79; haze, ii. 84, 89, 98; hop dimness, i. 221; maturing, ii. 93; pasteurising, i. 194, ii. 103–105; priming, i. 222; ropy, i. 219, ii. 133; turbidity, i. 194, 221, ii. 136
Beer-filters, i. 78, 79
Beer worts, i. 92, ii. 367, 368, 370, 371, 372; as nutriment for Torulaceæ, ii. 293; influence on reproduction of Torulaceæ, ii. 295; unsuitable for storing yeasts, ii. 163
Beer yeast, Belgian, plasma framework, ii. 115*; bottom formations, permanent cells, ii. 92,* 96, 107*; cytoplasm, ii. 109; descent from Mucor racemosus, ii. 78; fat content, ii. 127; gelatinous network, ii. 129, 130*; granules, ii. 112–116; influence of mechanical agitation, i. 63; Leignitz *a*, No. 405, ii. 393; Nos. 2, 18, 389, ii. 393; permanent cells, ii. 111,* 112,* 113,* 114*; sketch of observations by Cagniard-Latour, i. 12
Bees, foul brood in, i. 46
Beet, i. 202, 203, 218, ii. 507
Beggiatoa, breadth as a diagnostic character, i. 278, 279; cell contents, i. 33; culture, i. 278; morphology, i. 278, 279
Beggiatoa alba, behaviour under various life conditions, i. 279*; with scarcity of sulphuretted hydrogen, i. 279*; morphology, i. 279
alba, var. universalis, i. 280
media, morphology, i. 279
minima, morphology, i. 279
mirabilis, morphology, i. 279
roseo-persicina, i. 282
Bellalay cheese, peptone in, i. 243, 244
Benzaldehyde, ii. 269, 380
cyanhydrin, ii. 380
Benzamide, ii. 275
Benzene, i. 107, ii. 113; action of, on yeasts, ii. 181
Benzoate of ammonia. *See* Ammonium benzoate
Benzoic acid, i. 91, 161, ii. 275, 380; action of, on yeasts, ii. 181
Benzopurpurin, ii. 115
Benzoyl, ii. 269
Berkefeld filter, 78
Beryllium, ii. 33
Bignonia tomentosa, i. 309
Bilberry must, ii. 158
Bile, i. 229
Bimolecular optically inactive substance, i. 177
Bios, ii. 122; in yeast, ii. 152
Birch wine, ii. 100
Bismuth nitrate, action of, on yeasts, ii. 179
Black break, i. 193
rot, ii. 278
yeasts, ii. 302
Bladdery fermentation, ii. 134, 135
Blastoderma salmonicolor, ii. 301
Blastomycetes, ii. 76
Blood, lipase in, ii. 47
Blue grain of cheese, i. 117
spots of cheese, i. 117
Blueing of cheese, i. 117–119; of milk, i. 115–117
Bockbier wort, ii. 106
Böttcher cell, ii. 160*
Bog iron ore, i. 276
Boiling method, Roberts', i. 30, 53, 131, 132
of fruits and fruit-juices, i. 168
Boletus cyanescens, oxidising enzyme, i. 311
edulis, carbohydrates, ii. 28; casein-dissolving enzyme, ii. 46; cell wall, ii. 26; fongose, ii. 29; fungoid bodies, ii. 6*
luridus, blueing of, i. 311
Bolometric method, i. 113
Borax, i. 86, 109, 161, ii. 131; action of, on proteolysis, ii. 411
Boric acid, i. 86, 161, ii. 411; action of, on yeast, ii. 179
Botrytis, ii. 237; conidia-formation, ii. 41; rheotropism, ii. 44
cinerea, ii. 262; ammonia-producer, i. 235; appressoria-formation, ii. 45; behaviour to cæsium and rubidium, ii. 31; cellulose-dissolving enzyme, ii. 44; chemotropism, ii. 45; chitin in, ii. 28; conidial formation, ii. 41; influence of alkalies, ii. 32; insolation, ii. 43; intergrowth, ii. 5*; magnesium not essential for, ii. 32, 33; mechanical pressure of hyphæ, ii. 45; phosphorus in, ii. 37; zinc stimulant for, ii. 33
vulgaris, ammonia-producer, i. 235
Bottled beer, pasteurising, ii. 104

Bottom yeasts, ii. 82–87, 91*; gum in, ii. 128; modified to yield top fermentation, ii. 194, 195; No. 93, ii. 91*; pentosan in, ii. 129
Bouillie Bordelaise, ii. 173
Bouillon, preparation, i. 95
Bouillon-gelatin, preparation, i. 99
Bouquet, ii. 379
Bovista, ii. 26
Branching, false, i. 275*
Brandy, ii. 379, 380
Bread, behaviour of Claviceps purpurea in, ii. 73; black, as a culture medium, ii. 232; disease in, i. 135; influence of potato bacillus on, i. 135, 136; Mucors in, ii. 62
Bread flavour of beer, ii. 104
mould. *See* Penicillium glaucum
"Break" in wine, ii. 136, 137
Brettanomyces, ii. 297, 298
Breweries, germ content of air in, i. 77
Brewery handbooks, i. 93
water, biological examination of, i. 99
yeasts, tests for top fermentation, ii. 99
Briarea, description, ii. 219
Brie cheese, ii. 249, 256, 274
Bronze, ii. 281
Brood cell. *See* Gemma
Bryophyta, i. 22
Bryum, i. 270
Buckwheat and nitrogen, i. 288
Budding, influence of temperature on, ii. 192, 193, 210; in Monilia, ii. 330; in Mycoderma, ii. 306; in Oidium, ii. 336; in Saccharomyces apiculatus, ii. 315; in Torulaceæ, ii. 293, 300; of yeast cells, ii. 165, 204, 205, 206, 207, 211, 213, 214, 215
Budding fungi, ii. 8; and yeast, ii. 81; in ropy wine and beer, ii. 129
Buds, aggregation of, ii. 8
Buffon's system of generation, i. 3, 4
Burnt hay, i. 129, 130
Burton yeast, influence of strength of extract on reproductive capacity of, ii. 167
Butalanin, ii. 412
Butomus umbellatus, blueing of milk, i. 117; culture of Beggiatoa, i. 278
Butter, ii. 299, 336; aroma, i. 181; defects, i. 182; fishy (train oil), i. 182; from sterilised cream, i. 180; moulds of, ii. 47; oily, i. 182; pathogenic bacteria in, i. 160; sour cream, i. 180; sweet cream, i. 180; turnip-flavoured, i. 182
Butterwort, rennet in, i. 186; relation to Taette moelk, i. 216
Butyl alcohol, i. 145, 146, ii. 150, 276, 376; action of, on grape sugar, ii. 178
fermentation, i. 145; influence of age of seed and reaction of media, i. 148
Butyric acid, i. 145, ii. 150, 262, 276, 372, 378; action of, on yeasts, ii. 180; as a yeast poison, i. 188; in yeast, ii. 127; obtained by fermentation, i. 139; produced by Bac. lupuliperda, i. 128; by Micrococcus casei amari, i. 252; by Micrococcus gummosus, i. 218, 219; production of, ii. 297
Butyric acid bacteria, aromatic substances produced by, i. 147; in boiled milk, i. 251; theory of fermentation by, ii. 58
Butyric acid fermentation, equation, i. 270; excitation of, in stored-up granulose, i. 34
Butyric acid granulose, i. 139–153, 219, 251, 252
Byssus, ii. 39

Cacodyl, ii. 37
Cadaverin, i. 233
Cadmium, ii. 33
Cæsalpinaceæ, i. 263
Cæsium, ii. 31
Cagniard-Latour's theory of fermentation, i. 11, 12
Calcium, ii. 32–34
acetate, ii. 365
bisulphite, i. 85
borate, action of, on yeast, ii. 179
butyrate, ii. 276
carbonate, ii. 263, 276, 365, 371; utility of, in nitrification, i. 290
chloride, i. 94, ii. 349, 387, 410
citrate, ii. 266
formate, ii. 150
glycerate, ii. 150
glycerophosphate, ii. 150
lactate, i. 140, ii. 150, 276, 365
oxalate, ii. 30, 86, 262, 263, 264, 276
pectate, i. 151
phosphate, i. 94, ii. 345
tartrate, i. 140, 148
Callose, ii. 29
Camembert cheese, ii. 246, 275
Cane sugar, i. 94; behaviour of luminous bacteria with, i. 124; decomposition by Bacillus œdematis maligni, i. 140
Cantal cheese, i. 244, 245, 252
Cantharellus cibarius, cell wall, ii. 26
Cap, rubber, i. 76
Capric acid, ii. 372, 378
Caproic acid, ii. 372, 378
Caproyl alcohol, ii. 378
Caprylic acid, ii. 372, 378
Capsule bacillus, i. 31, 215
substance, i. 31, 32
Capsule-staining, i. 31, 32, ii. 130
Caragheen, i. 101
Carbohydrates as source of carbon for yeast, ii. 150; consumption of, by yeasts, ii. 151; gummy, from yeasts, ii. 127–130; influence on production of sporangia and zygospores, ii. 14, 15; mucinous, ii. 127; yielded by yeast nucleic acid, ii. 118
Carbol, ii. 409
Carbolic acid, action of, on bacteria, i. 87; on yeasts, ii. 181; as an antiseptic, i. 87; effect on Bac. coli commune and anthracis, i. 87; on Bacillus mesentericus ruber, i. 135; on formation of casease, i. 187
Carbon, carbohydrates a source of, for yeasts, ii. 151; sources of, ii. 148 *et seq.*
Carbon dioxide, i. 23, ii. 361, 362, 363, 364, 365, 368, 369, 370, 387; effect on bacteria, i. 142; exhalation, ii. 42; germicidal

power of, i. 85; produced by Allescheria Gayoni, ii. 272; by Mycoderma from alcohol, ii. 311 by Oidium lactis, ii. 337; by Saccharomycetes apiculatus from grape juice, ii. 321, 322; by Torulaceæ, ii. 296; by Willia anomala, ii. 214; by yeasts, ii. 341; from madder, ii. 342; from fermentation of yeast, ii. 410; from sugar, ii. 312, 404, 406; from wine must, ii. 326, 327*; from yeast, ii. 170, 171; in fermentation of saccharose liquids, ii. 360; influence of, on the xanthin bodies, ii. 412; on the yeast cell, ii. 178, 179; retarding effect of, ii. 295; yield from sugar, ii. 360
Carbon monoxide, ii. 387
Carbonic acid often means carbon dioxide, *which see*
Carlsberg bottom yeast, No. 1, action of carbon dioxide on, ii. 178; break, ii. 137; cell-form, ii. 86; description, ii. 203; film cells, ii. 93; habitat, ii. 184; network, ii. 129, 130*; variability of, ii. 189, 190, 197
Carlsberg bottom yeast, No. 2, ii. 203; action of tartaric acid on, ii. 180; cells, ii. 86; film cells, ii. 93; used for making good beer, ii. 196; variability of, ii. 189, 190
Carne pura, i. 164
Carnin, ii. 121, 412
Carnivorous plants, i. 231
Carnos, ii. 122
Carotin in Torula, ii. 300; in Monilia sitophila, ii. 301
Carpoasceæ, ii. 72, 218
Carpoasci, ii. 72, 218 *et seq.*
Carposporangial zygomycetes, ii. 48
Carpozyma, ii. 314
Carubinose, ii. 382
Casease, ii. 274; origin and activity, i. 186, 187, 231; production by Bacterium synxanthum, i. 109; by Sarcina rosea, i. 108; by Tyrothrix catenula, claviformis, distortus, filiformis, geniculatus, scaber, tenuis, turgidus, urocephalum, i. 245; secreted by bacteria, i. 108
Casein, action of Aspergillus niger on, ii. 274; decomposition by Mucors, ii. 62; digestion of, by yeast, ii. 411; dissolving enzyme, ii. 46; influence on lactic fermentation, i. 171; paranuclein, ii. 146; proportion in milk, i. 184; split up by lab, i. 184
Caseoglutin, i. 243
Cassage, i. 308
Cassure, i. 308
Catalase, ii. 277; occurrence of, in Torulaceæ, ii. 296, 297
Cell, absorption affinities for dye-stuffs, ii. 121; energy, ii. 42; in yeast film, ii. 88,* 89,* 90,* 91* 92*, 93; influence of lithium chloride, i. 36; influence of nutrition on form of, i. 28; influence of temperature, i. 28; plasmolysis in, i. 32
Cell contents, ii. 291, 292; structure of, i. 33
Cell division, i. 42, 43
Cell form as a characteristic, ii. 200; dependent on nutritive conditions, ii. 84; and on temperature, 88 *et seq.*; of Sacch. Ludwigi, ii. 100, 101
Cell forms in Dematium pullulans, ii. 282; in Monilia, ii. 330
Cell membrane, ii. 291; chemical analysis of, i. 211, ii. 30, 35, 39; chemical properties of, i. 30, 50; optical properties of, i. 30; permeability of, ii. 167, 168; resisting power, i. 50; stratification, ii. 106; thickening, ii. 106
Cell nucleus, ii. 10; chemical composition, ii. 120; fusion, ii. 14; effect of light, ii. 39–43; of pus cells, ii. 120
Cell wall, analysis, i. 30
Cellulose, ii. 270, 271, 365; dissolving enzyme, ii. 44; fungus cellulose, ii. 24, 25, 28; importance in nutrition physiology, i. 150; Omelianski's fermentation of, i. 149; reaction, i. 30, ii. 24; in the yeast cell wall, ii. 107; in yeast, ii. 404
Cellulosin, i. 148
Central body of cells, i. 33, 44
filament, ii. 115
Centrifuge, blueing of cheese by, i. 117
Centrosomes, ii. 10
Cephalothecium roseum, ammonium-producer, i. 235; change of arsenious acid by, ii. 37; gelatin-liquefaction, ii. 46; lignin, ii. 30
Cerealin, i. 205
Cerevisiæ, type, ii. 82, 87
Chætocladiaceen, ii. 49
Chalara mycoderma, ii. 335, 336; influence of insolation on, ii. 43
Chalk nutrient medium, i. 101
Chamberland filter, i. 77, 78
flask, ii. 161
Champagne, preparation of, ii. 137
Characters in Aspergillus, ii. 225–227; of yeasts, ii. 200
Charque, i. 164
Cheddar cheese, ripening of, i. 246
Cheese, ii. 281, 301, 302, 331, 336; aroma of clover, i. 246; bacterial flora of Swiss, i. 245, 246; bitter, i. 251; blind, i. 249; blue coloration in, i. 117; cause of puffy, i. 249; change in bacterial flora of ripening, i. 245; crude, i. 184; decomposition of lactose in making, i. 173; defects in, i. 248; fat in, i. 244; green coloration, i. 122; hindrances to ripening, i. 245; microscopy, i. 118; Nissler, i. 249; nitrogen compounds, i. 243 *et seq.*; normal pitting, i. 248; odour of, i. 246; oxalic acid as a decoloriser of red lead, i. 109; pigment bacteria in, i. 109; puffy, i. 249, 251; red coloration of, i. 108, 109; rhodonate compounds in, i. 109; rich, i. 184, 185; ripening of, i. 243 *et seq.*; skim, i. 184, 185; study of Cantal, i. 244
Cheese-makers' recipes, i. 250
Chemical composition of cell wall, i. 30
influence, effect of, on yeast, ii. 173–182
Chemotaxis, i. 40, 41, ii. 43

Chemotropism, i. 41, ii. 43
Cherries, ii. 324
Cherry brandy, ii. 372, 375, 380
Cherry-juice, ii. 105
Chicha, preparation of, i. 148
Chinese yeast, ii. 63, 66
Chitin, decomposition and occurrence, ii. 26, 27, 28, 44, 45; dissolving enzyme, ii. 44; in yeast, ii. 107
Chitosamine, ii. 27
Chitosan, ii. 27
Chitose, ii. 27
Chlamydomucor casei, ii. 62
oryzæ, dextrose, ii. 68
racemosus, chlamydospore germination, ii. 19*; oidia, ii. 18*; oidia germination, ii. 21*
Chlamydospores, ii. 11, 18, 291; formation, ii. 18; germination, ii. 20, 21
Chlorate of potash, repellent effect of, ii. 44
Chlorella protothecoides, pure plate culture, i. 103
vulgaris, pure plate culture, i. 103
Chloride, bacterial motion arrested by, i. 32
of lime, antiseptic, i. 85, 86. *See* Calcium chloride
of potassium, stimulating action on bacteria, i. 232. *See* Potassium chloride
Chlorine water, action of, on spores of Bacillus anthracis, i. 86
Chlorococcum (Cystococcus) humicola, pure plate cultivation, i. 103
Chloroform, i. 91, ii. 387, 390, 409, 410, 416; action on fermentation, ii. 350; on yeasts, ii. 405
Chlorophyll, action on assimilation of carbon dioxide, i. 23, 114; indispensability of iron, ii. 34
Chlorosphæra limicola, pure culture, i. 103
Chlorothecium saccharophilum, pure culture, i. 104
Chlor-zinc-iodide, ii. 24, 26, 107
Choanephoreæ, ii. 49
Cholera bacillus. *See* Vibrio choleræ Asiat.
Cholera-red reaction, i. 224
Cholesterin, ii. 127, 351
Cholin, i. 233, ii. 367, 412, 413
Chondrus crispus, i. 101
Chromatin, i. 33,* 34, 46; in nucleus, ii. 121
Chromatium, single flagella, i. 39, 111
Okenii, structure of cell, i. 33, 111,* 113,* 281*
Chromic acid, ii. 115
Chromogenic bacteria, i. 73, 105
Chromoparous, i. 105
bacteria, i. 105–110, 116
Chromophorous, i. 105
Chromophyll, i. 114
Chroococcus, i. 23
Chyme, i. 229
Chymosin, i. 185
Chytridiaceæ, motile spores, ii. 10; zoospores, ii. 12, 43
Cider, ii. 328, 329; ropy, i. 218; yeast, Wädensweil, ii. 133
Cilia, i. 37–39; histology of, i. 39–40
Cinnamic acid, action of, on yeasts, ii. 182
Circinella, ii. 53
Citrate of ammonium. *See* Ammonium citrate
of potassium. *See* Potassium citrate
Citric acid, ii. 150, 262, 265, 266, 267, 276, 297, 395; action of Mycoderma on, ii. 310; action of, on yeasts, ii. 180; decomposition by fission fungi, i. 240, 241; influence of, on the formation of daughter cells, ii. 8; organic food for Aspergillus niger, ii. 57; produced by Citromyces glaber and C. Pfefferianus, ii. 256, 259; by Penicillium luteum, ii. 259; from lactic acid by Saccharomyces apiculatus, ii. 323
Citromyces, ii. 246, 249, 255, 276; descriptive, ii. 219–221
citricus, ii. 256
glaber, acid-formation by, ii. 265; description, ii. 256
lacticus, ii. 257
oxalicus, ii. 257
Pfefferianus, acid-formation by, ii. 265; description, ii. 256, 257*
tartaricus, ii. 257
Citrus, ii. 265
Cladosporium, ii. 302
aeris, ii. 265
herbarum, ii. 278, 279, 280*; action of light on, ii. 288; conidia-formation, ii. 16; effect on cheese and eggs, ii. 281; on fermentation, ii. 280; glycogen content, ii. 123
Cladothrix, i. 27, 51, 272
dichotoma, false branching, i. 275*; gelatin pure culture, i. 276; morphology, i. 273; occurrence, i. 275, 276; plasmolysis, i. 32
odorifera, i. 277
Classification of bacteria, i. 69–74
Clathrocystis roseo-persicina, i. 282
Claviceps microcephala, influence of light on the length of the perithecial hyphæ, ii. 40
purpurea, chitin in, ii. 28; fongose, ii. 29; gelatin-liquefier, ii. 46; glycogen reserve, ii. 126; lignin, ii. 30; nuclei, ii. 10; sclerotium, ii. 73
Cleistocarp, ii. 73
Clostridium, i. 26, 47, 143
butyricum, cilia, i. 144*; form and size of spores, i. 47*, 48, 49, 52*; influence of oxygen on spore-formation, i. 48; length of generation, i. 144; morphology, i. 143, 144*; plasmolysis, i. 33; pure culture, i. 50; size, i. 27; spore-formation, i. 47,* 52*; structure of cilia, i. 39, 40
fœtidum, i. 47; chemical activity, i. 144, 145
fœtidum lactis, odour of, i. 147
Pasteurianum, i. 269–271
polymyxa. *See* Granulobacter polymyxa
Clover cheese, aroma of, i. 146
Cobalt, ii. 35

Cobalt sulphate, ii. 349
Coccobacteria septica, i. 70, 71, 208
Coccus, i. 25
Cochineal insects, ii. 316
Cocoa bean as culture medium, ii. 236
Coefficient of reproduction. *See* Reproductive capacity
Coffee berries, ii. 240; substitute, ii. 122
Cognac, ii. 372, 375, 379, 380
Cohn's classification of bacteria, i. 69, 70
Cold for preserving meat, i. 163
Cold-loving bacteria, i. 58, 59
Cold-torpidity, i. 40, 60
Collidin, i. 232, ii. 380, 413
Colon bacillus. *See* Bact. coli commune
Colonies, liquefactive, i. 103; solid, 103; zooglœa, ii. 1
Colour alteration, ii. 121
change of wort, ii. 92
Colouring bacteria, i. 105–110
Colouring-matter, ii. 10, 11; protective against light, ii. 43; yielded by species of Allescheria, ii. 257, 258; Aspergillaceæ, ii. 275; Aspergillus, ii. 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242; Citromyces, ii. 256, 257; Dematium pullulans, ii. 283; Monilia, ii. 331, 332, 333, 334; Mycoderma, ii. 309; Mycosphærella, ii. 280; Oidium, ii. 336, 338; Penicillium, ii. 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255; red yeasts, ii. 299–302; Saccharomycetes, ii. 288; Torula, ii. 292; Torulaceæ, ii. 293
Columella, ii. 11; in Mucor javanicus, ii. 68; M. mucedo, ii. 52*; M. pyriformis, ii. 53; M. racemosus, ii. 52, 53*; M. Rouxii, ii. 64*; M. spinosus, ii. 53
Commissariat bread, i. 136
Composition of bacterial cell, i. 35
Compressed vegetables, i. 168
Conferva, i. 270
Congo red, ii. 107
Conidia, ii. 11, 219 *et seq.*, 221, 222,* 228,* 229,* 231,* 232, 233,* 234, 235,* 236,* 237, 238,* 239, 240, 241,* 242, 243,* 244, 245, 246, 247,* 248, 249, 250,* 251,* 252,* 253,* 254, 255, 256, 257,* 258, 278, 279,* 280,* 281, 282, 283, 284,* 288, 330, 331, 332,* 333,* 334, 337, 338; chain of, ii. 15*–16*; fructification, ii. 15*–17; influence of light on formation, ii. 41; order of succession, ii. 15–16
Conidiophores, ii. 15–17, 220 *et seq.*, 228,* 229,* 230, 231,* 232, 233,* 234, 235,* 236,* 237, 238,* 239, 240, 241,* 242, 243, 244, 246, 247,* 248, 249, 250,* 251,* 252,* 253,* 254, 255, 256, 257,* 258, 279, 280,* 333*
Coniferæ, mycorrhiza, i. 271
Coniferin, ii. 45; fission of, ii. 269
Conserves, manufacture of, introduced by Appert, i. 11, 161, 168
Contact attraction, ii. 56
Convallamarin, ii. 269
Copper, i. 122, ii. 128, 129, 131; action of yeasts on, ii. 174, 175
Copper phosphates, production of, by yeasts, ii. 174
salts, effects of yeasts on, ii. 173–175
sulphate, action of, on yeast, ii. 405
as a fungicide, ii. 173; as a destroyer or stimulant of yeast, ii. 173, 174
Coprinus, formation of pileus, ii. 40; heliotropism, ii. 39
lagopus, heliotropism, ii. 39
niveus, influence of ultra-red on, ii. 39, 40; migration of glycogen, ii. 126
stercorarius, glycogen in, ii. 6; pileus-formation, ii. 41
Copulation cell, ii. 13
Core yeast, ii. 132
Coremia, ii. 244, 248
Coremium, ii. 17
Corium, i. 204
Cork disease, ii. 238, 281
Cormophytes, i. 22, ii. 1, 3
Cormus, i. 22, ii. 1
Corn mildew. *See* Puccinia grammarum
Corned beef, i. 165, 166
Coronation, ii. 292
Corrosive sublimate, i. 84, ii. 338, 349; action of, on yeast, ii. 174, 179
Cotton plant and atmospheric nitrogen, i. 264
Cotton-seed oil, ii. 271
Cotton-wool filter, i. 76
Counting chamber, Wolfhügel's, i. 96, 97,* 102
Cow-dung, germ-content in, i. 155
Cow-tree, i. 139
Cranberry jam as culture medium, ii. 231
Crassulaceæ, ii. 262
Cream, aroma of, i. 181; artificial souring, i. 180
Crenothrix, i. 27, 51, 272
Kühniana. *See* C. polyspora
polyspora, blueing of cheese, i. 118; culture on bricks, i. 276; morphology, i. 272, 273,* 274*; multiplication, i. 273, 274; occurrence, i. 275, 276; plasmolysis, i. 32, 33
Cryptococcus, ii. 76
fermentim, ii. 202
glutinis, ii. 299
guttulatus, ii. 211. *See also* Saccharomyces guttulatus
vini, ii. 314
Crystalloids in vacuoles of yeast, ii. 111
Crystals in cells, ii. 292
Cultivation of yeast, ii. 159–164
Cultures, comparison of, i. 103, 104
pure, i. 28, 35, 66, 96, ii. 85
Cupuliferæ, mycorhiza, i. 271
Curd, i. 184, 185; after-warming of, i. 250, ii. 105
Curdling of milk, i. 184
Currant wine, sulphurous acid used for, ii. 147
Currants, ii. 324
Cuvée, ii. 137
Cyanhydrin, ii. 270
Cyanophil, ii. 121

Cynara scolimus, lab-formation, i. 186
Cytase, ii. 270
Cytoplasm, i. 34, ii. 10, 109, 114; nitrogenous constituents, ii. 119–123
Cytosin, ii. 119

Dactylium oogenum, ii. 281
Dædalea quercina, phosphoric acid extraction from wood, ii. 37
Dahi, occurrence of typhoid bacillus, i. 156
Dahlia variabilis, i. 310
Daphnia, i. 42, ii. 215
Daphnidæ, ii. 75
Darwin's evolution theory, i. 8
Dates, ii. 239
Daughter cells, forms of, i. 42, 44
Decay, i. 224
Decoloration of wine, i. 239
Degeneration of yeast, ii. 197
Dematium, ii. 8, 184, 215, 286, 287, 302, 332, 335
  casei, ii. 283, 284, 285
  pullulans, ii. 281, 282, 283, 284*, 285*; behaviour to ammonia salts, ii. 16; hyphæ, ii. 29; intergrowth of, ii. 5; yeast conidia, ii. 16
Denecke's spirillum. *See* Spirillum tyrogenum
Denitrification, i. 236–238
Desiccation, effect of, on Saccharomyces apiculatus, ii. 320; on Torulaceæ, ii. 297; of spores, ii. 22
Desmobacteria, i. 70
Dextran, i. 208, 211, ii. 129
Dextrin, ii. 15, 150, 261, 271, 272, 399, 400, 401, 402, 403; as a stimulant of fungi, ii. 43; behaviour with β- and γ-Amylomyces, ii. 65; with Mucor Rouxii, ii. 61; fermentation of, ii. 61, 296, 332, 399; ferments of, ii. 216, 217; from starch by Bac. suaveolens, i. 147; influence on Sacc. Marxianus, ii. 191; saccharification by Mucor, ii. 61
Dextrinomyces, ii. 151
Dextrolactic acid. *See* Lactic acid
Dextrose, ii. 15, 260, 261, 266, 268, 269, 270, 271, 272, 320, 403; action of Sacch. Hansenii on, ii. 209; as a culture medium, ii. 228; as a nutrient, ii. 167; attacked by Allescheria Gayoni, ii. 269; fermentation of, by Saccharomyces apiculatus, ii. 320, 321, 323, 324; by Torulaceæ, ii. 301; from melecitose, ii. 268; ferments of, ii. 203, 206, 208, 209, 210, 211, 214, 215, 216, 217; influence on reproductive capacity of yeasts, ii. 167; influence on Sacc. Ludwigii, ii. 191; non-ferments of, ii. 214; not fermented by Zygosaccharomyces Barkeri, ii. 209, 210
Diabetic sugar, ii. 382
Diagnostic table for bacteria, i. 72
Diastase, ii. 228, 302, 380, 389, 403; as source of nitrogen for yeasts, ii. 155; discovery, i. 17; formation by Aspergillaceæ, ii. 260, 262; Aspergillus niger, ii. 46, 47; A. oryzæ, ii. 65; Bacille amylozyme, i. 147; Bacillus anthracis, B. Fitzianus, megatherium, i. 148; B. mesentericus vulgatus, i. 135; ramosus, i. 148; suaveolens, i. 148; subtilis, tetragenus, i. 148; Granulobacter butylicum, i. 145; Mucor, ii. 63; M. Rouxii, ii. 65; M. alternans, ii. 65; Penicillium glaucum, ii. 46, 47; Vibrio choleræ Asiaticæ, i. 148; secreted by Aspergillaceæ, ii. 260
Diatomaceæ, i. 22
Diethylarsine, ii. 37, 275
Differential staining of spore-bearing bacterial cells, i. 50, 51; of tubercle bacilli, i. 51
Diffusion fields, i. 125
Digallic acid, ii. 271
Digestio, i. 10
Digestion, i. 229
Digitalin, ii. 269
Dimargiris, description, ii. 219
Dionæa muscipula, i. 232
Dioxyacetone, ii. 381
Dioxy-γ-ketone, ii. 363, 364
Dioxypropionic aldehyde, ii. 362, 364
Diplococcus, i. 26,* 42
Disaccharides, ii. 381
Discomycetes, ii. 29, 73, 218
Disodium phosphate, ii. 348
Dispira, description, ii. 219
Dispora caucasica, spore-formation, i. 48
Division in one direction, i. 42
  in three directions, i. 43
  in two directions, i. 43
  longitudinally, i. 42
Division of the nucleus, i. 48
  transversely, i. 42
Dothidea puccinoides, ii. 282
  ribesia, ii. 282
Double refraction of cell wall, i. 31
Drinks imparting stability, ii. 103, 104
Drosera longifolia, i. 232
  rotundifolia, i. 231, 232
Drosophila cellaris, i. 305
  funebris, i. 305
Dry rot, i. 88, ii. 37
Drying of vegetables and fruits, i. 168
Dulcitol, ii. 150; action of Sacch. Hansenii on, ii. 209
Dyes, behaviour of endospores towards, i. 50, 51

Echinobotryum atrum, ii. 281
Edam cheese, pitting in, i. 248; use of Streptococcus hollandicus for, i. 216
Eel, i. 234
Egg-albumen, derivation of glycogen from, ii. 125
Eggs, preservation of, i. 166; putrefaction of, i. 234, ii. 281
Elæagnus angustifolius, nodule-formation by, i. 263, 264, 265, 266
Elaphomycetes, affinity, ii. 218
Electricity, action on bacteria, i. 57, 61; utilisation in the food-stuff industries, i. 58

Elements essential for Eumycetes, ii. 31, 38
Ellipsoideus type, ii. 83, 84
Emericella, description, ii. 219
Emmenthaler cheese, appearance of, after warming, i. 250; bacteria in, i. 245, 246; nitrogenous compounds of, i. 243; normal pitting, i. 248
Empusa muscæ, ii. 48
Emulsin, ii. 45, 268, 269, 395, 399
Endoblastoderma, ii. 332
liquefaciens, ii. 382
mycoides, ii. 382
Endomyces decipiens, ii. 74*
Endospores (endogenous spores), behaviour towards dyes, i. 50; change and condensation of cell contents by plasmolysis, i. 32; formation of, i. 48, 49; formation in Mucor mucedo, ii. 11; germination, i. 52–56; resisting power, i. 49, 50, 135
Endosporium, i. 52, ii. 13, 21
Endotryptase, ii. 355; properties of, and action in yeast-division and in fermentation, ii. 408–415; yeast, ii. 355
Ensilage, i. 201–203
thermometer, i. 201
Entomophthoreæ, ii. 49
Enzymes, i. 17; action of carbolic acid on, i. 87; action of digestive, on fermentation, ii. 352; alcoholic fermentation by, ii. 58, 59; casein-dissolving, ii. 46; cellulose-dissolving, ii. 44, 45; diastatic, ii. 46, 47; distribution, ii. 358; fat-splitting, ii. 47; luminous bacteria as test for, ii. 125; gelatin-liquefying, ii. 46; glucoside-splitting, ii. 45; in Aspergillaceæ, ii. 259; in Monilia, ii. 334; in Oidium, ii. 337; in yeasts, ii. 340–358; in yeast juice, ii. 186; inverting, of Mucors, ii. 61; oxidising (oxydases), i. 308–312; proteolytic, ii. 46; variations of, in yeasts, ii. 190, 191
Enzymology, application of the bacteria filter, i. 78
Epiplasma, ii. 123
Equisetinæ, i. 22
Ergosterin, ii. 127
Ergot of rye, ii. 6, 26
Ericaceæ, mycorhiza, i. 271
Erysipelas, i. 62
Erysiphe aceris, iodine coloration of, ii. 123
Erythrite, effect of, on zygospore production, ii. 15
Erythritol, ii. 150
Erythrocellulose, ii. 108
Erythrodextrin, ii. 68
Erythrophil, ii. 121
Esmarch tubes, i. 102
Esters, ii. 214, 215, 372, 375, 377, 379, 380; action of, on proteolysis, ii. 411; produced by Mucors, ii. 61; by Saccharomyces apiculatus, ii. 324, 328; Torulaceæ, ii. 296; and by Willia anomala, ii. 214
Ether, ii. 127, 132, 135, 351; as an antiseptic, i. 89; for cold sterilisation of food, i. 89
Ethyl acetate, ii. 377
alcohol, i. 136, 137, 140, ii. 150; as an antiseptic, i. 89; food for acetic acid bacteria, i. 89; influence on anthrax spores, i. 89; production by fission fungi, i. 137, 195, 216, 217, 240, 249
caprinate, ii. 379
caprylate, ii. 379
carbonic anhydride, ii. 362
pelargonate, ii. 379
succinate, ii. 379
Euaspergillus, ii. 220
Eucalyn, ii. 391
Euinvertase, ii. 384
Eumycetes, cells, ii. 8; classification, ii. 17; colour, ii. 10; definition, i. 22, ii. 1, 2, 3; septation, ii. 3; spores, chemical analysis, ii. 22
Euonymus europæus, ii. 212
Eurostose, ii. 122
Eurotiella, ii. 257
Eurotin, ii. 260
Eurotiopsis, ii. 219, 221; action on alcohol, ii. 272
Gayoni, ii. 257, 269
Eurotium, ii. 220, 221, 226
Aspergillus flavus, ii. 233
Aspergillus glaucus, ii. 230, 231
Aspergillus medius, ii. 232
Gayoni, ii. 261
glaucum, ii. 230
herbariorum, ii. 230
insigne, ii. 255
malignum, ii. 242
oryzæ, ii. 227, 242
repens, ii. 230, 232; conidia, ii. 16; conidiferous hyphæ, ii. 13
rubrum, ii. 232
Exoasci, ii. 73, 74
Exoascus, ii. 288
deformans, ii. 288
pruni. *See* Taphrina pruni
Exospore, ii. 11, 15
Exosporium, i. 53, ii. 13, 21

Fæces cerevisiæ, i. 10
vini, i. 10
Faro, 196
Fat-colouring matter, i. 107–108, 283
Fat-decomposing enzyme, ii. 47. *See also* Lipase
Fats, ii. 292, 365, 367, 368, 369, 373, 378; breaking up of, i. 153; decomposition of, by mould fungi, ii. 50, 51; importance of, for yeasts, ii. 126, 127; rancidity of, ii. 152
Fatty acids, ii. 370, 378, 379, 413
manures, i. 151
Fehling's solution, ii. 118, 124, 128, 129, 131
Fermentation, action of, on milk, i. 184; activity, ii. 59; by Saccharomyces apiculatus, ii. 320–324; definition of, i. 1, 18, 20; flasks, i. 159, 251; lactic—*see* Lactic; fibre, ii. 138; of snuff, i. 129; of tobacco,

i. 128; optically active organic compounds produced by, i. 174; organisms, botanical position of, i. 21; physiology defined, i. 21; test of milk, i. 251; theories, i. 10, ii. 340 *et seq.*; theory of Cagniard-Latour (vitalistic), i. 11; of Gay-Lussac, i. 10; of Kützing, i. 13; of Nägeli (molecular physical), i. 16; of Pasteur, i. 16; of Stahl, i. 10; of Traube, i. 17
Ferro-gelatin, medium for sulphuretted hydrogen bacteria, i. 225
Ferrous sulphate, ii. 349; action of, on yeast, ii. 405
Fibrin, action of Aspergillus niger on, ii. 274; of yeast on, ii. 409; digestion of, ii. 46; digestion of, by endotryptase, ii. 411
Ficus carica, lab-formation, i. 186
Figs, ii. 239, 332
Filicinæ, i. 22
Filmjölk, i. 216
Films, ii. 87 *et seq.*, 291, 294, 301, 307–309, 331, 333, 335, 336, 338; annular, ii. 88; as characteristics, ii. 200; developmental stages of yeasts, ii. 88 *et seq.*
Filter, Chamberland's, i. 77, 78; efficiency of, i. 76; v. Breyer's, i. 78
Filter-paper as substitute for gypsum block, ii. 96
Filtration of drinking water, i. 77–78
Fish poisons, i. 234
Fish, red, ii. 10
Fission fungi. *See* Schizomycetes
Fixing plasmolysed cells, i. 33
Flagella, i. 37–39
Flavour altered by pasteurising, ii. 104
Flax retting, i. 152
Flocculence in making pressed yeast, ii. 93
Flour, sclerotium of Claviceps purpurea in, ii. 73
Flowering rush and blueing of milk, i. 117; culture of Beggiatoa with help of, i. 278
Fluorescent bacteria, i. 121, 122
Fluorides, action on mycoderm, ii. 313
Fodder, green pressed, i. 201; sour, i. 202
Fongine, ii. 24
Fongose, ii. 29
Foodstuffs from yeast, ii. 122
Foot and mouth disease, milk as carrier of, i. 156, 160
Form, theory of constant, i. 69
Formaldehyde, ii. 338, 387, 390, 410; action of, on fermentation, ii. 350 (formalin)
Formalin, action of, on Mycoderma, ii. 313; on typhus bacillus, anthrax bacteria, and Staphylococcus pyogenes aureus, i. 90; on yeast cells, i. 90; as an antiseptic, i. 89; for preserving cultures, i. 103
Formate of ammonium. *See* Ammonium formate
of calcium. *See* Calcium formate
of potassium. *See* Potassium formate
of sodium. *See* Sodium formate
Formic acid, i. 137, 140, 147, 159, ii. 150, 262; action of, on yeast, ii. 180; influence of, on wine yeasts, ii. 326, 328; produced in alcoholic fermentation, ii. 370; by mycoderms, ii. 311; by Saccharomyces apiculatus, ii. 324; by yeast, ii. 92, 171
Formol, i. 89
Fouh-ling, ii. 28
Foul brood in bees, i. 46
Fountain planes, i. 286
Fractional culture, i. 98
Fragmentation, i. 44, ii. 110
Freudenreich-Hansen flask, ii. 163,* 164
Frog-spawn in sugar manufacture, i. 208
Frohberg yeast, ii. 203, 393, 397, 402; action of acetic acid on, ii. 180; of benzoic acid, ii. 181; influence of age of sowing on reproductive capacity of, ii. 168; asparagin on same, ii. 166; of carbon dioxide on, ii. 178; of temperature on period of generation, ii. 165
Fructification in Monilia, ii. 330; influence of light on, ii. 41; sporangial, dependent on external conditions, ii. 14, 15
Fructification organ, ii. 2
Fructose, ii. 150, 151, 377, 381; alcohol-formation from, by β- and γ-Amylomyces, ii. 65, 66; by Mucor Rouxii, ii. 63; fermentation of, ii. 332, 333; by Torulaceæ, ii. 296
Fruit, intramolecular respiration of, ii. 58; occurrence of Saccharomyces apiculatus on, ii. 324, 328
juices, i. 169; fermentation of, ii. 380
sugar, ii. 383
Fruits as the habitat and breeding-place of yeasts, ii. 183–188
Fry's ensilage, i. 201
Fuchsine, i. 267, ii. 121
Fuh-ling, ii. 28
Fumago, ii. 302
salicina, ii. 282
Fumaric acid, ii. 150
Fungi, i. 22, ii. 3; as excitants of fermentation, i. 18, 21; cell nucleus, ii. 10; centrosomes, ii. 10; imperfecti, ii. 19, 78, 80; in mines, ii. 39
Fungine in mines, ii. 39
Fungoid bodies, ii. 6; mucin, ii. 128
Fungus cellulose, ii. 24, 25, 26; hypha, ii. 2
Furfural, ii. 129, 151, 377, 380; action of, on yeasts, ii. 181
Furfurol, ii. 29
Fusarium, ii. 29
heterosporium, action of light on, ii. 40
Fusel oil, ii. 371, 372, 375, 376, 377, 378, 413

Galactane, ii. 129
Galactans, ii. 383
Galactoaraban, ii. 383
Galactodendron americanum, i. 139
Galactone, i. 231
wine, i. 231

Galactosazone, ii. 394
Galactose, ii. 15, 150, 268, 273, 382, 383, 394, 395, 396, 398; action of Sacch. Hansenii on, ii. 171, 209; fermentation of, ii. 332, 335; by Sacch. Soja, ii. 208; Saccharomycodes Behrensianus, ii. 210, 211; by Torulaceæ, ii. 296; non-ferment of, ii. 214; not fermented by Willia anomala I., ii. 214; nor by Willia anomala III., ii. 215
Galactoxylan, ii. 383
Galium verum, i. 108
Gall-nuts as a nutrient medium, ii. 238
Gallate of ammonium. *See* Ammonium gallate
Gallic acid, i. 14, ii. 238, 271
Gamete, ii. 13
Gammelost, ii. 256, 285; participation of species of Mucor in ripening of, ii. 62
Garlic, ii. 63, 67; odour, ii. 37
Gas carbonum, ii. 360
sylvestre, ii. 360
Gas-pressure, influence of, on bacteria, i. 65
Gastric juice, action of, on yeast cells, ii. 113; artificial, ii. 120
Gay-Lussac's fermentation theory, i. 10
Geaster formicatus, examination of, for cellulose, ii. 26
Geasterin, ii. 26
Gelatin, ii. 275; acted on by endotryptase ii. 408, 409, 411, 413; by yeasts, ii. 413; as culture medium, ii. 213; as a nutrient medium for yeast, ii. 408; liquefaction by Aspergillaceæ, ii. 273, 274; by Oidium lactis, ii. 337; by Saccharomyces apiculatus, ii. 324; by Torula, ii. 301; by Torulaceæ, ii. 297; liquefying enzyme, ii. 46; tubes, i. 102. *See also* Wort gelatin *and* Must gelatin
Gelatinous network, ii. 129, 130,* 131
Gelose, i. 100
Gemmæ, ii. 11, 18, 48, 64, 68, 87, 283; formation, ii. 18, 19
Gemmating mycelium, ii. 7; from Chlamydospores, ii. 21; in Mucor, ii. 7
Generatio equivoca, Needham's demonstration, i. 3; spontaneous, i. 3
Generation, period of, i. 44, 45
Gentianose, ii. 399; fission of, ii. 268
Gentiobiose, ii. 268, 399
Geotropism, i. 65
Germ, generative power of, i. 45
Germ-content of air, Frankland and Petri's estimate of, i. 76; percentage in air of breweries, i. 77; determining, i. 96
Germination of endospore, i. 52–56
Germs, destruction of, i. 79–94, 156–162
Gherkins, ii. 336
Giant cells, ii. 85, 86, 214
colonies, ii. 214, 293, 301, 306, 307, 317, 330
Ginger-beer, i. 66, 195–198; yeast, i. 195–198
Glairine, i. 281
Gleditschia, root-nodules wanting in, i. 263
Gliocladium, description, ii. 219
penicilloides, ii. 246, 255
Gliscrin (mucus), i. 219
Globule bacteria, i. 69
Globulins, i. 184, ii. 345
Glucacetase, ii. 371
Glucase, i. 145, ii. 389
Glucomyces, ii. 151
Glucosamine, ii. 27
Glucosazone, ii. 394
Glucose, ii. 150, 151, 271, 363, 365, 368, 371, 377, 381, 382, 383, 385, 390, 391, 392, 394, 395, 396, 397, 398, 399; action of Sacch. Hansenii on, ii. 171; action on proteolysis, ii. 411; as a chemotactic stimulant, ii. 43; on Mucor mucedo, ii. 43; on Mucor Rouxii, ii. 43; Rhizopus nigricans, ii. 43; behaviour of photobacteria with, i. 124; decomposed by Granulobacter saccharobutyricum, i. 146; by sunlight, i. 20; conversion of achroocellulose into, ii. 108; of erythrocellulose into, ii. 108; and of yeast nucleic acid into, ii. 118, 119; fermentation of, ii. 331, 332, 335, 337, 360; by Saccharomyces apiculatus, ii. 321; by Torulaceæ, ii. 296; glycogen formed from, ii. 125; produced by yeast, ii. 404, 406; from mycosin, ii. 27; from yeast gum, ii. 128, 129
Glucoside-splitting Eumycetes enzymes, ii. 45
Glucosides, ii. 380; fission of, ii. 269, 270
Glutamic acid, ii. 345, 412
Glutamine as glycogen-former, ii. 125
Glutaminic acid, ii. 150
Glutin, digestion of, by endotryptase, ii. 411
globules, ii. 86; action of acetic acid on, ii. 130, 131
Glutopeptone, ii. 273
Glycerate of calcium. *See* Calcium glycerate
of potassium. *See* Potassium glycerate
Glyceric acid fermented by Bac. ethaceticus, i. 137, 178
Glycerin, i. 136, 137, 239, ii. 25, 43, 58, 61, 125, 127, 150, 311, 317, 333, 345, 365, 366, 367, 368, 369, 376, 378, 381, 413; action on living organisms, ii. 350; on proteolysis, ii. 410, 411; as a source of citric acid, ii. 267; yielded by Allescheria Gayoni, ii. 272
Glycerol, action of Sacch. Hansenii, ii. 171, 209
Glycerophosphate of calcium. *See* Calcium glycerophosphate
Glycerophosphoric acid, ii. 145, 367, 413
Glycerose, ii. 382
Glycocoll, i. 258, ii. 275, 349; action of, on proteolysis, ii. 410, 411
Glycogen, ii. 6, 91, 108, 292, 345, 346, 363; absent in asporogenic cells, ii. 191; present in Mycoderma, ii. 305; in red Torulaceæ, ii. 300; in yeast, ii. 405, 406
Glycogenase, properties of, ii. 405
Glycoheptose, ii. 382
Glycyrrhicin, ii. 270

Gold-leaf, penetration of, by fungus hyphæ, ii. 45
Gomme de sucrerie, i. 208
Gomphonema, branched gelatinous stalk, i. 212
Gonidia, ii. 11
Gooseberries, ii. 324
Gorgonzola cheese. *See* Stracchino
Gossypium, relation of, to atmospheric nitrogen, i. 264
Gossypose, ii. 397
Gouda cheese-rind, i. 247
Graham bread, i. 136
Granules, ii. 112, 114,* 115, 331; coloration of, ii. 113; contents of, ii. 113, 114; fat content of, ii. 114, 127
Granulobacter, genus, i. 145; storing of granulose by, i. 34
butylicum, i. 143, 145, 146
lactobutyricum, chemical activity of, i. 146; morphology, i. 146; rancidity of fats, i. 146, 152
polymyxa, involution forms, i. 29*; morphology, i. 146; spore-formation, i. 52
saccharobutyricum, i. 146
Granulose, i. 34, 145; inaction of Chlamydomucor oryzæ, ii. 68
Grapes, Saccharomyces apiculatus on, ii. 324, 325; Sacch. ellipsoideus on, ii. 325
Grass flavour, i. 181
Gravity, influence on growth, i. 65
Green bacteria, i. 122
coloration of cheese, i. 122
manuring, i. 260
pressed fodder, i. 201
racking, ii. 135, 136
Ground water bacterium, i. 137
Gruyère cheese, i. 243
Guaiacol, ii. 277
Guaiacum, tincture, i. 311
Guanylic acid, ii. 118, 120
Guanidin, ii. 275
Guanin, i. 247, ii. 119, 120, 121, 412
Gueuse lambic, i. 196
Guignardia Bidwelli, ii. 278
Gum-arabic, ii. 383
Gummose (mucus), i. 218
Gummosis of sugar-beet, i. 215, 218
Gums, ii. 383
Gymnoasceæ, affinities, ii. 72, 218
Gymnoascus, ii. 220
Gypsum, elimination from water by Spirillum desulfuricans, i. 226
block cultures, ii. 86, 96,* 130

Habitat of Saccharomycetes, ii. 183–188
Hadromal, ii. 45
Hadromase, ii. 45
Hands, disinfection of, i. 89
Hansen method of single-cell culture, ii. 159–164
Hansenia, ii. 201, 315; description, ii. 209
Hanstein's aniline violet, ii. 107, 108
Hard cheese ripening, i. 246
Hay, ii. 331; air-dried, i. 130, 200; brown, i. 130, 151, 199, 200; burnt, i. 129, 130, 200; germ content, i. 155
bacillus. *See* Bacillus subtilis
Head in beer, ii. 134–138
Heat liberated during alcoholic fermentation, ii. 348; resistance of spores to, ii. 22
Heath, mycorhiza, i. 271
Helicin, fission of, ii. 269
Heliotropism, ii. 39
Helobacteria, i. 47
Hemi-albuminose, ii. 349
Hemiasci, ii. 79
Hemi-basidii, ii. 80
Hemicelluloses, i. 150, ii. 28
Hepatinæ, i. 22
Heptyl alcohol, ii. 379
Hesperidin, ii. 269
Heterodera Schachtii, i. 214
Heterogenesis, i. 3, 7
Hexoses, i. 147, ii. 382; as food for Saccharomyces apiculatus, ii. 319
Hibiscus, ii. 230
Hides, fermentation of, i. 204
Hippophaë, i. 263, 264
Hippuric acid, decomposition by bacteria, i. 257, 258, ii. 275
Histidin, ii. 274, 412
Histology of cilia, i. 39
Honey, ii. 401
wine, ii. 145
Hop resins, ii. 93; action of, on yeasts, ii. 180, 181
tannins, effect of, on yeasts, ii. 181
Hops as a culture medium, ii. 232; spontaneous heating of, i. 128
Hordeum distichum nudum, i. 145
Hormiscium cerevisiæ, ii. 202
Hormodendron cladosporioides, i. 167, 279, 281
Horny matter, solution of, by fungi, ii. 46
Horse-dung, nitrogen liberated by, i. 237
House-fly disease, ii. 48
Household cheese, bacteria in, i. 245
Hunger-torpidity, i. 40
Hydration, i. 224
Hydrochloric acid, ii. 26, 117, 124, 128, 348, 392, 395; action of, on micro-organisms, ii. 179; on proteolysis, ii. 411; effect on enzymes, ii. 261
Hydrocollidine, i. 233
Hydrocyanic acid, ii. 269, 380, 387, 391; action of, on enzymes, ii. 349; on yeasts, ii. 341
Hydrofluoric acid, ii. 387; action of, on micro-organisms, ii. 179; action of, on vital activity of yeast, i. 192; as an antiseptic, i. 86, 191; prevention of inversion, i. 213
Hydrogen, ii. 363, 364, 368, 373, 378, 416; effect of, on bacteria, i. 142
Hydrogen peroxide, ii. 297, 362, 363; as an antiseptic, i. 86; for control of water filter, i. 87; produced in sterile urine exposed to sunshine, i. 61
Hydrogenase, ii. 364, 415

Hydrokinone, action of, on yeasts, ii. 180
Hydrolysing enzyme of yeast, ii. 126; processes, i. 192, 193
Hydrolysis of glycogen, ii. 125, 126
Hydrotropism, ii. 13
Hydroxyl, ii. 322
Hydroxylamine hydrochloride, ii. 350
Hypha, ii. 2
Hyphomyces, ii. 11
Hyphomycetes, ii. 6, 10
Hyposulphites, ii. 41
Hypoxanthin, ii. 119, 120, 412

Ice, bacteria in, i. 164
Ilex aquifolium, ii. 206
Illumination and respiration quotient, ii. 57; clarification of beer by light, ii. 136; influence of light on vegetation, ii. 39
Immunity, i. 234, 235
Indian meal, detection of arsenic in, ii. 38
Indican, i. 119
Indicators for nutrient media, i. 101
Indiglucin, i. 119
Indigo bath, i. 121
  blue, i. 119
  brown, i. 120
  carmine, ii. 416
  fermentation, i. 119, 120; enzyme of, i. 120
  maladies, i. 121
  red, i. 120
  rubin, i. 120
  white, i. 119
Indigo-sulphuric acid, i. 143
Indigofera, i. 119
Indole, i. 224
  nitroso-, i. 224
  reaction, i. 224
Infection threads, i. 267; coloured in sections, i. 267
Infusions, ropy, i. 218
Infusoria, i. 2, 69
Infusum foliorum digitalis, ropy, i. 218
  senega, gelatinised, i. 218
Inosinic acid, ii. 120
Inositol, ii. 150
Insects as carriers of yeasts, ii. 325
Intercalary gemmation, ii. 18; growth, ii. 5
Intestinal formation of toxic nitrites by Bact. coli commune, i. 236; gases, i. 151; putrefaction, i. 224, 229
Intramolecular respiration, ii. 57, 60, 61
Inulase, ii. 302; species of Aspergillaceæ secreting, ii. 270
Inulin, ii. 208, 216, 270, 383; action of Mucor Rouxii on, ii. 66; derivation of alcohol from, by β-Amylomyces, ii. 65
Inulose, ii. 150
Invert sugar, ii. 368, 435; content of molasses in, i. 213; decomposition of, i. 20; influence on the crystallisation of cane sugar, i. 212
Invertase, ii. 260. 269, 302, 321, 337, 341, 351, 352, 381, 384–388, 392, 398; produced by Aspergillus niger, ii. 268; by Bacillus fluorescens liquefaciens, B. megaterium, i. 213; B. subtilis, i. 134; red Kiel bacillus, i. 213; Bacterium gelatinosum betæ, i. 213; Chlamydomucor oryzæ, ii. 68; by Leuconostoc mesenteroides, i. 211; by Monilia candida, ii. 331; by Proteus vulgaris, i. 213; by Torulaceæ, ii. 296; by Willia anomala, ii. 214; not produced by Penicillium Duclauxii, ii. 268
Invertin, i. 211, ii. 384
Invisible heat rays, i. 114
Involution forms in Schizomycetes, i. 28, 29; in yeasts, ii. 83 *et seq.*
Iodide solution, i. 30, ii. 29, 123, 128
Iodine in iodide of potassium, ii. 107
Iodoform, i. 90, 91
Iodosulphuric acid, ii. 24, 29
Ions, ii. 33
Irish moss, i. 101
Iron, ii. 34, 35; organic compounds of, ii. 35
  alum, ii. 109
  bacteria, decomposing power, i. 276; food-supply, i. 276; importance in water examination, i. 276, 277; morphology, i. 272–275; physiology, i. 275–277
Iron-phosphorus compounds, ii. 35
Isaria, ii. 254
Isoamyl alcohol, ii. 376, 378
Isobutyl alcohol, i. 148; ii. 376
Isobutylene glycol, ii. 368
Isodulcite, sporangia-formation, ii. 15
Isoheptyl alcohol, ii. 379
Isolactose, ii. 396
Isolation by means of chemotaxis, i. 40
Isoleucin, ii. 378
Isolichenin, ii. 29
Isomaltose, ii. 391, 396, 400
Isomannose, ii. 382
Isopropyl alcohol, ii. 376

Jacquemase, ii. 415, 416
Jalapin, ii. 269
Johannisberg yeast, action of copper sulphate on, ii. 173, 174; No. II., description, ii. 205; limits of temperature, ii. 165; No. 2, sporulation experiments with, ii. 193, 195
June flavour, i. 181
Juniper berry juice, ii. 181
Jusée, i. 206

Karthaus yeast, ii. 326, 328
Karyokinesis, i. 44, ii. 10, 110
Karyoplasm, ii. 110
Kefir, Kefyr, Kephir, ii. 208, 209; granules, i. 66, 118
Ketotriose, ii. 381
Koch's plate cultures, i. 99
Koji, i. 248, ii. 214, 253
  fungus. *See* Aspergillus oryzæ
Koumiss, ii. 208, 298
Kräusen-glutin, ii. 132
Kreolin, i. 88

Lab, ii. 274, 297; activity of, i. 184, 185; occurrence of, in nature, i. 184, 186; -producing bacteria, acting in boiled milk, i. 185, 186
Laccase, chemical activity, i. 309; manganese content of, ii. 36
Laccol, i. 309
Lacquer, Japanese, i. 309
Lactacidase, ii. 345, 364
Lactalbumen, i. 184
Lactarius, oxydase, i. 311
piperatus, manganese in, ii. 35
Lactase, ii. 269, 302, 394–396; formation by Saccharomyces Kefyr, i. 125
Lactate of ammonium. *See* Ammonium lactate
of calcium. *See* Calcium lactate
of potassium. *See* Potassium lactate
of sodium. *See* Sodium lactate
Lactic acid, ii. 150, 260, 276, 331, 345, 348, 363, 364, 365, 370, 372, 387, 390; action of Mycoderma on, ii. 310; of Oidium lactis on, ii. 337; of Saccharomycetes apiculatus on, ii. 323; as a fixing medium, i. 33; as a glycogen-former, ii. 125; bottom fermentation, i. 173; content in yeast mash, i. 191; effect on Bacillus subtilis, i. 134; on enzymes, ii. 261, 268, 269; ethylidene, i. 175, 178; fermentation, i. 175, 178; in Edam cheese, i. 119; in ordinary beers, i. 195; in ropy infusions, i. 218; influence of the nutrient conditions on variation, i. 178, 179; lævo-, i. 175, 178, 179; obtained from glucose and lactose, i. 20; para-, i. 175, 178; preparation for technical purposes, i. 190; produced from glucose and lactose, i. 20; from succinic, malic, and citric acids by Saccharomyces apiculatus, ii. 323; from sugars, ii. 262; production of, by Bacterium peptofaciens, i. 231; by Mucor Rouxii, ii. 63
Lactic acid bacteria, artificial souring of cream with, i. 180; as starter, i. 180; co-operation in causing rancidity of fat, i. 152; culture media for, i. 103; dilution method used for, i. 246; discovery of, i. 170; effect of hop resins on, i. 95; function in ripening of Emmenthal cheese, i. 246; in artificial souring of mash, i. 189; in beer, i. 194; in distilling, brewing, and preparation of wine, i. 193; in intestine, i. 229; in preparing fodder, i. 201; in sauerkraut fermentation, i. 203; in wine, i. 194; involution forms, i. 29; nutrient medium for, i. 100; occurrence of, i. 171
Lactic acid fermentation, enzyme of, i. 173; equation of, i. 172; influence of casein and phosphate, i. 173; for salted gherkins, i. 168; of bark liquor, i. 205; of beer, i. 194; of brown hay, i. 199; of cream, i. 180; of plumping soak, i. 204; of wine, i. 193; with Bacillus diatrypeticus casei, i. 249; with Leuconostoc mesenteroides, i. 211; with Micrococcus casei amari, i. 252
Lactic aldehyde, ii. 362, 363
anhydride, ii. 362
Lactoglycose, ii. 382
Lactomyces, ii. 151, 262
inflans caseigrana, ii. 289, 294, 295, 297
Lactoprotein, i. 184
Lactose, ii. 150, 151, 202, 206, 208, 210, 211, 214, 215, 268, 273, 301, 302, 320, 382, 392; action of, on proteolysis, ii. 410, 411; action of Sacch. Hansenii on, ii. 171, 209; as a culture medium, ii. 208; behaviour of Sacch. Kefyr to, i. 125; of Mucor Rouxii to, ii. 65; decomposition of, by bacteria, i. 172; by heat, i. 140; in cheese, i. 248; in sunshine, i. 20; fermentation of, ii. 295, 332, 333, 335, 337; by Allescheria Gayoni, ii. 272; by Bacillus amylozyme, i. 147; occurrence of, in Torulaceæ, ii. 296; influence on zygospore-formation, ii. 15
Lævulan, ii. 128, 383
Lævulose, i. 20, ii. 272, 383; action of Mycoderma on, ii. 312; as glycogen-former, ii. 125; behaviour of Mucor Rouxii towards, ii. 65; derived from gentianose, ii. 268; influence on zygospore-formation, ii. 15; fermented by Allescheria Gayoni, ii. 269, 272; by species of Mucor, ii. 65; by Saccharomyces apiculatus, ii. 320; by S. Soja, ii. 208; by Saccharomycodes Behrensianus, ii. 211; by Saccharomycopsis capsularis, ii. 211; Schizosaccharomyces pombe, ii. 216; by Willia anomala I., ii. 214; by Willia anomala II., ii. 214; by Zygosaccharomyces Barkeri, ii. 209, 210
Lamarck's development theory, i. 8
Lambic, i. 195, 196
Laurent's solution, ii. 294
Lauric acid, ii. 372
Lead, effect of, on yeasts, ii. 175
acetate, action of, on yeasts, ii. 174
Leather as a culture medium, ii. 232
Lecithin, i. 233, ii. 127, 345, 349, 368, 413
Leguminous nodules, i. 19; artificial formation of, i. 264; bacterial growth in plant, i. 266, 267; bearing on fertility of soil, i. 269; connection between nodule-formation and growth of plant, i. 263; discovery of, i. 261; formation of, i. 261; functions of, i. 263; increase of nitrogen-yield due to, i. 262; nature of, 268*; section of, 262,* 263
Leguminous plants, behaviour towards nitrogen, i. 259–268
Lemon-juice, ii. 67
Leprosy bacillus, resistance to decolorising agents, i. 51
Leptobryum, i. 270
Leptothrix, i. 27, 51, 197
buccalis, i. 146, 147
ochracea, i. 275, 276
Leucin, ii. 274, 345, 378, 380; as a metabolic product of Tyrothrix species, i. 245; decomposition by Bacillus mycoides, i.

235; extracted from yeast cells, ii. 121; in higher fungi, ii. 46; occurrence of, in ripe cheese, i. 243; origin and decomposition of, i. 187, 224; produced by yeast, ii. 408, 409, 412, 414
Leucomaines, i. 233
Leuconostoc mesenteroides, i. 209,* 210*; behaviour towards sugars, i. 211; conversion of mucinous envelope of, i. 212; double staining of, i. 210, 211; effect of calcium chloride, i. 211; of nutrient media, i. 210, 211; invertin produced by, i. 211; occurrence in sugar refineries, i. 211, 212; physiology, i. 210; resisting power of, i. 210
Leuconuclein, ii. 146
Liebig's decomposition theory, i. 14; theory of fermentation, ii. 340
Light, action of, on invertase, ii. 387; on maltase, ii. 390; decomposition by, i. 20; influence on bacteria, i. 60–63; on butter and cheese, i. 152, 153; on development of Eumycetes, ii. 39–43; on germination of spores, ii. 40; on spore-formation, i. 49; torpidity, i. 40
Light rays replaced by heat rays, ii. 41
Lighting gas, arsenic in, ii. 38; effect of, on bacteria, i. 142
Lignin, ii. 30
Limane, i. 284, 285
Limburger cheese, odour, i. 147; ripening, i. 246
Lime, ii. 365; importance of, for yeast, ii. 140–143; in reference to "head"-formation, ii. 143
Linin, i. 34, ii. 121
Linseed, ii. 29
Lipara monacha, ii. 290
Lipase, ii. 47, 271, 367, 368, 378
Lipochrome, i. 107–108, 283
Lipocyanine, i. 107
Liporhodine, i. 107
Lipoxanthine, i. 107
Liquefactive colonies, i. 103
Liquefiable solid media, i. 99
Liquide Pictet as an antiseptic, i. 85
Raulin, ii. 36
Lithium, ii. 32
chloride, effect on cells, i. 36
salts as tests for the rotatory power of lactic acids, i. 179
Liver, glycogen of, ii. 123
Locomotion of bacteria, i. 37–40
Logos yeast, ii. 203, 394, 396, 402, 403; action of acetic acid on, ii. 180; influence of age of sowing on reproductive capacity and on reproductive power, ii. 168; of carbon dioxide on, ii. 178; of yeast water on reproductive capacity, ii. 167
Long rods, i. 25, 26
Longitudinal division, i. 42
Luciferin, i. 126
Luminosity of fish, i. 123 *et seq.*; meat, i. 123; sea, i. 123, 126; small marine animals, i. 126
Lupeose, ii. 383
Lupines, nitrogen increase, i. 268; nodules of, i. 267
Lupulin granules, ii. 86
Lycopodinæ, i. 22
Lysin, ii. 274, 412
Lysol, i. 88

Macassar, fish, ii. 10
Macrococci, i. 272
Macrogonidia, i. 272
Macrosporium verrucolosum, ii. 281
Madder, fermentation of, ii. 342
Magnesia, ii. 140, 141; as an essential food, ii. 32, 33
Magnesium carbonate, influence on nitrification, i. 290
chloride, ii. 405
phosphate, ii. 345
sulphate, i. 147, 311, 349, ii. 94
Magnetism, action on bacteria, i. 58
Magnusia, descriptive, ii. 218
Maize, ii. 389; behaviour of nitrogen to, i. 203; diastase in, ii. 155; pentosans in, ii. 151
meal, detection of arsenic in, ii. 38
Malase, i. 310
Malate of ammonium. *See* Ammonium malate of potassium. *See* Potassium malate
Malic acid, ii. 150, 276, 369; action of Mycoderma on, ii. 310; action of, on Saccharomyces apiculatus, ii. 319; action of, on yeasts, ii. 180; decomposition of, by fission fungi, i. 240; influence on respiration, ii. 57; produced by Saccharomyces apiculatus, ii. 322; stimulating action on bacteria, i. 232
Malt, ii. 371, 383, 388; gum content of, ii. 131; pentosans in, ii. 151
culms as food for yeast, ii. 156
extract, influence on reproductive capacity, ii. 167
sugar, ii. 388
wort gelatin, i. 146
Maltase, ii. 302, 321, 381, 388–391, 392; secreted by Allescheria Gayoni, ii. 269; by Aspergillus niger, ii. 268; by Penicillium glaucum, ii. 268
Maltodextrins, ii. 399
Maltol, action of, on yeast, ii. 181
Maltomyces, ii. 151
Maltopeptone, ii. 145
Maltose, ii. 150, 273, 345, 369, 381, 389, 391, 399, 400; action of Sacch. Hansenii on, ii. 171, 209; attacked by Mycoderma, ii. 311, 312; behaviour of luminous bacteria to, i. 124; decomposition of, by Granulobacter saccharobutyricum, i. 146; fermentation of, ii. 331, 333, 335, 337; by Allescheria Gayoni, ii. 272; by Granulobacter butylicum, i. 145; by Torulaceæ, ii. 296, 301; ferments of, ii. 202, 208, 211, 216, 217; fission of, ii. 268; formation of dextrolactic acid from, i. 20; influence on reproductive capacity of yeasts, ii. 167; non-ferments of, ii. 206, 210, 214, 215; zygospore-formation, ii. 15

Mandelic acid, ii. 270; dissociation of the optically inactive, i. 178
Manganese, a substitute for iron in the iron bacteria, i. 276; in relation to laccase, ii. 35, 36
sulphate, ii. 349; action of, on yeasts, ii. 405
Manganous chloride, action of, on yeast, ii. 179
Manna, ii. 399
Mannan, ii. 128, 382
Mannite, fermentation of, i. 241; by Bacillus ethaceticus, i. 137; glycogen-formation favoured by, ii. 125; produced by Penicillium glaucum, i. 242; produced during the mucinous fermentation of sugar, i. 242; zygospore-formation favoured by, ii. 15
Mannitol, ii. 150; action of, on proteolysis, ii. 411; action of Sacch. Hansenii on, ii. 171, 209
Manno-nonose, ii. 381
Mannose, ii. 382, 383; fermentation of, ii. 335; by Saccharomyces apiculatus, ii. 320; by Sacch. Soja, ii. 208
d-, ii. 216; alcohol formed from by β- and γ-Amylomyces, ii. 66; by Mucor Rouxii, ii. 66; yielded by achroocellulose, ii. 108; by yeast gum, ii. 129
Mannotriose, ii. 399
Manuring, green, i. 260
with ammonia salts, i. 293, 294
Marienthal yeast, ii. 93
Marine phosphorescence, i. 124–126
Mars, i. 196
Marsh's arsenic test, ii. 38
Matière glycogène, ii. 123
Mazun, ii. 208, 298, 299
Mead, properties of, ii. 145
Meat extracts, purity of, ii. 122; sterilising, i. 83
phosphorescent, i. 124; preservation of, i. 163–166; storage of, in cold chambers, i. 163
Meat-extract bouillon, preparation, i. 95, 96
Meat-poisoning, i. 233
Mechanical shock, influence of, on bacteria, i. 63–65
Melampyrite, ii. 150
Melecitase, secreted by Aspergillus niger, ii. 268
Melibiase, ii. 268, 391–394
Melibio-glucase, ii. 392
Melibiosazone, ii. 394
Melibiose, ii. 268, 296, 382, 391, 392, 393, 394; action of β-Amylomyces on, ii. 66; of Mucor Rouxii on, ii. 65
Melicitose, ii. 296, 399; decomposition of, ii. 268
Melitose, ii. 391, 397
Melitriose, ii. 268, 383, 391
Membrane, lignification of, ii. 32; stratification of, ii. 106
Mercaptan, i. 226
Mercury chloride (corrosive sublimate), i. 84, ii. 387, 390, 416
Merismopedia, i. 23
Merismopedium, i. 43
Metabiosis, i. 66, 239
Metacellulose, ii. 24
Metallacter, i. 70
Metaphosphoric acid, ii. 146
Meteors as carriers of organisms, i. 8
Methyl alcohol, i. 107, ii. 150, 276, 351, 375, 380, 413; action of, on grape sugar, ii. 178
Methyl glucoside, ii. 208
a-, behaviour of Mucor Rouxii to, ii. 65; fermentation of, ii. 333
β-, behaviour of β- and γ-Amylomyces to, ii. 66; fermentation of, ii. 333, 335
d-, ii. 208
Methyl green, ii. 121
violet, ii. 130
Methylene blue, ii. 107
Methylglyoxal, ii. 365
Methylindole, i. 224
Methylpropylcarbinol, ii. 378
Methylpyromeconic acid, ii. 181
Methyl-salicylates, ii. 271
Methyluracyl, ii. 119, 120
Mica plate, ii. 43
Microascus, description, ii. 218
Microbacteria, i. 69
Microbe, i. 2
Micrococcus, i. 26, 27,* 69; of bitter milk, i. 252; bitter flavour of milk, cream, and butter, i. 252; formation of butyric acid by, i. 252
Micrococcus acidi lactis, peptonising enzyme, i. 172
acidi paralactici, i. 67; formation of paralactic acid by, i. 178; mixed culture with Anthrax bacillus, i. 67
agilis, locomotion, i. 39
agilis citreus, locomotion, i. 39
ascoformis, proteolytic enzyme, i. 230
aurantiacus, orange-yellow colouring-matter, i. 110
candicans, ammonia-producer, i. 235
casei amari, i. 252
chlorinus, i. 122
erythromyxa, colouring-matter, i. 107
flavus, ammonia-producer, i. 235
Freudenreichii, i. 215
gelatinogenus, gelatinises infusions, i. 218
gummosus, i. 218
lactis acidi, i. 172
ochroleucus, yellow colouring-matter (protective), i. 110
Pflügeri, phosphorescence of slaughtered animals, i. 124; pure culture, i. 123; spectrum of light from, i. 126
phosphoreus, i. 123
prodigiosus, colour, mutability, i. 106; coloration of milk, i. 106; effect of electricity on, i. 57, 58; effect of light on, i. 62; elementary decomposition of bacterial cell, i. 35; lab formed by, i. 186; lactic acid formed by, i. 171;

nitrogen in, i. 35; occurrence in saturation scum, i. 213; peptonising enzyme, i. 106, 230; quantitative selective power, i. 36
Micrococcus radiatus, sensitiveness to vibration, i. 64
ramosus, proteolytic enzyme, i. 230
rhodochrous, red colour of, i. 107
roseus, ammonia-producer, i. 235
saprogenes vini I., decoloration power, i. 239
saprogenes vini II., decoloration of wine, i. 239; morphology, i. 239
Sornthali, pitting of cheese, i. 249
tetragenus (mobilis ventriculi), cilia (locomotion), i. 39; fat-splitter, i. 153
tetragonus, morphology, i. 26*
ureæ, urea ferment, i. 255
ureæ liquefaciens, gelatin-liquefier, i. 255; urea ferment, i. 255
violaceus, violet colouring-matter, i. 121
viscosus, i. 219
Micro-fermentation, Lindner's, ii. 66
Microgonidia, i. 272
Micrography, i. 2
Microscope, compound, i. 2
Microsomata, Microzyma, i. 7, 8
Micro-spectrum, i. 40
Microzyme theory of Béchamp, i. 7
Mikrosol, action on Mycoderma cerevisiæ, ii. 313
Mildew, i. 241
black, ii. 281
fungus, ii. 123
Milk, ii. 299, 302, 335; arsenic in, ii. 37; as a carrier of foot-and-mouth disease, i. 156; as a carrier of infectious diseases, i. 155, 156; as a carrier of scarlet fever, i. 156; bitter, i. 251; blue coloration of, i. 115–117; coagulation of, i. 187, ii. 274, 275; condensed, i. 161, 162; curdling of, i. 184; curdling during thunderstorms, i. 187; Dematium pullulans, ii. 284; detection of gas-producing bacteria in, i. 159; effect of Torula on, i. 294; electrical treatment of, i. 58; germ content of, i. 154, 155; germ content after the Soxhlet treatment, i. 158; nitrogenous constituents of, i. 184; pasteurisation of, i. 161; preservation of, i. 154–162; red coloration of, i. 108–109; remedies for blue coloration of, i. 117; ropy, i. 215; soapy, i. 217; spontaneous coagulation, i. 187; sterilisation by boiling, i. 156; after the Neuhauss, Grönwald, and Öhlmann method, i. 159; yellow colouring of, i. 109
Milk casein, iron in, ii. 35
mould, ii. 335
sludge, i. 154
Milk of lime as an antiseptic, i. 87; as an egg-preservative, i. 167; effect on typhus and cholera bacteria, i. 87
Mimosaceæ, i. 263
Mineral antiseptics, i. 84
nutrients, ii. 31
Mines, fungi in, i. 139
Miso, i. 247, 248
Mitosis, ii. 110
Mitotic division of nucleus, i. 44
Mixed cultures, i. 67, 143
pickles, i. 168
sowings, ii. 98, 99
Molasses, nitric fermentation of, i. 213, 238; preparation of arrack from, ii. 67; sugar-extraction from, ii. 62
Molecular movement, Brownian, i. 37, ii. 115
Molecular-physical theory of Nägeli, i. 16
Molybdate of ammonia, ii. 37
Monas, i. 25, 69
Okenii. *See* Chromatium Okenii Warmingii, i. 111,* 281*
Monascus purpureus, ii. 11, 257
Monilia, ii. 8, 285, 287, 288, 293; affinities, ii. 330, 336, 338, 339; polymorphism in, ii. 330
albicans, description and properties, ii. 334, 337
candida, ii. 207, 301, 381, 384, 395, 397, 403; description, morphology, and properties, ii. 9*, 330, 331, 334, 335, 342, 358; effect of shocks on, i. 64; occurrence in tobacco fermentation, i. 129
fructigena, cellulose-dissolving enzyme, ii. 45
javanica, description and properties, ii. 333
sitophila, ii. 301, 330, 397; description and properties, ii. 333,* 334
variabilis, ii. 330, 394, 397, 403; description, morphology, and properties, ii. 332,* 333
Monomolecular, optically inactive substances, i. 176
Monomorph, ii. 19
Monomorphism, i. 28, 70
Monopodial branching, ii. 4
Monopodium, ii. 4
Monospora, ii. 75, 76; definition, ii. 202; description, ii. 215
cuspidata, ii. 75; description, ii. 215
Morchella esculenta, ii. 26
Morel, cholesterin in, ii. 127
Mortierella Rostafinskii, ii. 53
van Tieghemii, gemmæ, ii. 19; spores, ii. 13
Mother cells, alteration in form of, i. 46, 47
yeast, i. 189
Mother of vinegar, analysis, i. 297–298; nature, i. 14; significance of its appearance in vinegar fermentation, i. 306, 307
Mould films, ii. 90
Mucedineæ, ii. 6
Mucic acid, ii. 129
Mucilage from trees, ii. 5
Mucin, i. 27, 32, 129
Mucinic acid, ii. 150
Mucinous substances, ii. 345
Mucor, ii. 29, 288; casein-degradation by, ii. 62; ester-like products, ii. 61; enzyme in, ii. 46; fermentations, ii. 59; rheotropism, ii. 44; snuff-manufacture, ii. 62; species, pathogenic, ii. 51; suspensors, ii. 51

Mucor alternans, budding mycelium, ii. 10; diastatic enzyme, ii. 63, 69; raffinose-, saccharose-, trehalose-fermentation, ii. 61; sporangiophore, ii. 51, 53
ambiguus, budding mycelium, ii. 10
amylomyces Rouxii, in Chinese yeast, ii. 64
aspergilloides, ii. 53
Cambodja, ii. 395; in Chinese yeast, ii. 66
casei, ii. 62
circinelloides, budding mycelium, ii. 10; diastatic enzyme formed by, ii. 63; extraction of saccharose from molasses, ii. 62; formation of glycerin by, ii. 61; formation of succinic acid by, ii. 61; intramolecular respiration, ii. 59; ratio between alcohol and carbon dioxide, ii. 60; sporangiophore, ii. 51, 53
corymbifer, action of cæsium on, ii. 31, 32; of potassium on, ii. 32; of sodium on, ii. 32; action of, on arsenic, ii. 37; importance of magnesium, ii. 32, 33; mycosis, ii. 51, 52; sporangiophore, ii. 51
erectus, azygospore-formation, ii. 14,* 52; alcohol-yield, ii. 60; behaviour towards saccharose, ii. 61; budding mycelium, ii. 10; intramolecular respiration, ii. 59; sporangiophore, ii. 51
flavidus. *See* Sporodinia grandis
fragilis, budding mycelium, ii. 10; sporangiophore, ii. 51, 53
javanicus, ii. 395; behaviour with gelatin, ii. 46; sporangiophore, ii. 51
mucedo, alcohol-yield, ii. 60; ammonia-producer, i. 235; as a source of carbon, ii. 61; behaviour with arsenic, ii. 37; behaviour with glucose, ii. 43; budding mycelium, ii. 8; chemotropism, ii. 43; chitin content, ii. 28; cholesterin, ii. 127; effect of citric acid on, ii. 8; in eggs, ii. 281; filamentous mycelium, ii. 10; formation of succinic acid, ii. 61; fructification, ii. 41; gelatin-liquefier, ii. 46; germinating spore, ii. 3*; heliotropism, ii. 39; intramolecular respiration, ii. 61; lignification, ii. 30; mycelium swellings, ii. 19; occurrence, ii. 42; oxalic acid as a final metabolic product, ii. 142; sensitiveness to yellow light, ii. 39; sporangiophore, ii. 51, 52*; sporangium, ii. 12*; thallus, ii. 2*; tobacco-fermentation, ii. 62; zygospore-formation, ii. 13*
mucilagineus, sporangium, ii. 51*
nigricans, lignification, ii. 30
pusillus, mycosis, ii. 51; sporangiophore, ii. 51
pyriformis, acid produced by, ii. 265; citric acid produced by, ii. 53; columella, ii. 53
racemosus, alcohol-yield, ii. 60; ammonia-producer, i. 235; behaviour with arsenic, ii. 37; and with saccharose, ii. 61; budding mycelium, ii. 8; chitin content, ii. 28; chlamydospores, ii. 53*; diastatic enzyme, ii. 63; importance of iron for, ii. 34, 35; insolation, ii. 42; intramolecular respiration, ii. 61; inversion of lactose, ii. 61; mycosis, ii. 51; relation between alcohol and carbon dioxide, ii. 61; sporangia, ii. 41; sporangiophore, ii. 51; in tobacco, ii. 62
Mucor Rouxii, ii. 397, 403; action on gelatin, ii. 46; columella, ii. 64*; diastatic enzyme, ii. 65; in dough, ii. 71; invertin, ii. 65; mycelium-colouring, ii. 64; saccharose as a source of carbon, ii. 51; sporangiophore, ii. 51, 53; starch-fermentation, ii. 69
septatus, mycosis, ii. 51
spinosus, behaviour with saccharose, ii. 61; budding mycelium, ii. 10; intramolecular respiration, ii. 59; sensitiveness to alcohol, ii. 60; sporangiophore, ii. 51, 53
stolonifer. *See* Rhizopus nigricans
tenuis, azygospore, ii. 14, 53*; filamentous mycelium, ii. 10; sporangiophore, ii. 51
vulgaris, cellulose absent, ii. 26
Mucor yeast, ii. 78
Mucoraceæ, ii. 19, 298; cellulose absent in, ii. 26; glycogen content, ii. 123; sporangia, ii. 49
Mucoreæ, morphology and systematic position, ii. 50
Mucormycosis, ii. 51
Mucus formation, i. 208, 210, 219; from trees, ii. 100
Mucus threads, i. 267
Munich lager beer yeast, ii. 82
Muscarine, i. 233
Musci, i. 22
Must, ii. 299, 366, 371, 373
Must-gelatin, i. 100, ii. 160
Mustard, fixation of nitrogen by, i. 264
Mutability, i. 28, 70
Mutation. *See* Variability
Mycacanthococcus cellaris, spores, i. 51
Mycelicide, action on Mycoderma cerevisiæ, ii. 313
Mycelium, ii. 2; colour in Mucor javanicus, ii. 68; in M. Rouxii, ii. 64, 68; development of, ii. 3; septated, ii. 3; typical, ii. 3, 5
hair, ii. 5
threads, ii. 5
Mycetes, ii. 3
Mycetide, ii. 29
Mycetozoa, i. 22, ii. 123
Mycin, ii. 25
Mycŏderma, ii. 20, 90, 286, 287, 288, 291, 293, 303–313, 377, 413; acid-production and destruction, ii. 309–311; action of insolation on, ii. 42; Brownian motion, ii. 115; budding in, ii. 8, 306,* 307; filamentous, ii. 7; film-formation, ii. 307,

308,* 309; giant colonies, ii. 306, 307*; granules, ii. 112; influence of chemical agents on, ii. 312; of heat on, ii. 312; membrane, ii. 304, 305; morphology of cells, ii. 304, 305*; occurrence on grapes, ii. 325; pure cultures, ii. 304; vacuole enclosures, ii. 151
Mycoderma aceti. *See* Bacterium aceti
cerevisiæ, i. 12, ii. 34, 303; acids produced by, ii. 310; action of antiseptics, ii. 313
cucumerina, ii. 303
humuli, ii. 300, 301, 303; mortal temperature, ii. 104
lebenis, ii. 311, 312
Pasteurianum. *See* Bacterium Pasteurianum
rubrum, ii. 300, 303; mortal temperature, ii. 104
sp., ii. 153
vini, ii. 153, 286, 303, 312; barium injurious to, ii. 34; calcium not essential for, ii. 34; magnesium not essential for, ii. 32; potassium not essential for, ii. 31; strontium injurious to, ii. 34
Mycology, i. 21; museum, arrangement, i. 104
Mycomycetes, ii. 3, 10, 12; development of mycelium, ii. 3, 5
Mycoplasma, i. 266, 267
Mycoprotein, i. 35
Mycorhiza, i. 271
Mycosin, ii. 26, 27
Mycosis, ii. 51, 232
Mycosphærella, ii. 278, 281
Tulasnei, ii. 278, 279, 280*
Mycotetraedon cellare, spine-thickening of arthrospores, i. 51
Mycothrix, i. 42
Myrosin, ii. 271
Myxomycetes, i. 22

Nägeli's physico-molecular theory, i. 16
Nail bacteria, i. 47
Natto, i. 247, 248
Nematode disease, i. 214
Nematospora, definition, ii. 202; description, ii. 215
coryli, description, ii. 215
Nepenthes Mastersi, i. 232
Network, gelatinous, ii. 118
Neuridin, i. 233
Neurin, i. 233
Newskia ramosa, unilateral gelatinisation, i. 212
Nickel, ii. 35
Nicotin, i. 129
Nissler cheese, i. 249
Nitrate of bismuth in distilling, i. 191, 219
Nitrates in brewery water, ii. 154; no use for must yeast, ii. 154; reduction of, i. 236; retarding effect, i. 32; significance of, for Chlamydomucor oryzæ, ii. 68
Nitric acid, action of boiling, on yeast juice, ii. 129; action on albumen, i. 35; fermentation of molasses, i. 213, 238; and of tobacco, i. 238; proportion in rain-water, i. 261; reduction to nitrogen in mixed cultures, i. 68
Nitrification as a physiological process, i. 288–294
Nitrifying bacteria, assimilation in the dark, i. 291; division, i. 289; formation of wall saltpetre, i. 293; interrelation between carbon assimilation and nitrogen, i. 292, 293
Nitrite, behaviour of Chlamydomucor oryzæ with, ii. 68; formation from nitrates by Bac. ramosus and other bacteria, i. 236; formation in intestine by Bacterium coli commune, i. 236, 237
Nitrite-agar, i. 291
Nitrites, ii. 154, 331, 349
Nitrobacter, i. 291
Nitrobacteria, chemical activity of, i. 289, 290; morphology, i. 291; nutrient solutions for, i. 291
Nitrogen, ii. 267, 349, 370; accumulators, i. 259; circulation, i. 253, 270, 271; content of yeast membrane, ii. 108; effect on bacteria, i. 142, 143; hunger, i. 259; in alcoholase, ii. 353; in yeast juice, ii. 409; liberation, i. 235–238; taken in by bacteria, i. 19, 35, 259–271
Nitrogenous substances in bacilli, ii. 34; in yeast, ii. 121
Nitrosobacteria, chemical activity of, i. 290
Nitrosococcus brasiliensis, morphology, i. 290
Nitroso-indol, i. 224
Nitrosomonas africana, europæa, japonica, javanica, i. 290
Nitrous acid, ii. 416; influence on fermentation, ii. 348
Nitzschia, i. 270
Nodule bacteria, i. 264; development of, i. 265, 266, 267; distinctive species of, i. 265; occurrence of, i. 266, 267
Nonyl alcohol, ii. 379
Nostoc, i. 23
Nuclear fungi, ii. 73
Nuclease, ii. 275
Nucleic acid, ii. 118; stains for, ii. 121
Nuclein, action on, by endotryptase, ii. 411; base, ii. 117, 120; carbohydrate, ii. 118, 119; fixation of colour, i. 34; formation of, ii. 119, 120; in the granules, ii. 114; phosphorus content, ii. 37
Nucleoalbumens, ii. 345
Nucleoproteids, ii. 118, 275
Nucleus, ii. 292, 331; division of, i. 44, ii. 10; in red Torulaceæ, ii. 300
Nukamiso, i. 248
Nutrient bouillon, i. 95
fluids, Jaksch's, i. 255; Pasteur's, i. 94; potash content, ii. 31; respiration quotient of, ii. 57; van Tieghem's, ii. 41
gelatin, i. 99; from fish bouillon, i. 124

Nutrient media, alkalinity of, i. 102; for luminous bacteria, i. 125; importance of, in connection with the action of light on bacteria, i. 61; influence on fructification, ii. 15; liquefiable solid, i. 99; silica and chalk, i. 101
Nutrition, influence of, on bacteria, i. 133–134
Nuts as a culture medium, ii. 232

Oak, ii. 210, 382
Oakwood, phosphoric acid from, ii. 37
Oat, fixation of nitrogen by, i. 264; smut in, ii. 79
Object-marking, ii. 160, 161
Œnanthic ether, ii. 379
Œnanthol alcohol, ii. 379
Œnanthylic acid, ii. 372
Œnomel, ii. 145
Oidia-formation, ii. 18
Oidium, ii. 201, 285, 331, 332, 336, 337; descriptive, ii. 336
albicans, ii. 334, 336, 337; behaviour with gelatin, ii. 46
lactis, ii. 332; affinity, morphology, and properties, ii. 336,* 337, 338; behaviour with gelatin, ii. 46; influence of sunlight, ii. 43; influence on cheese-ripening, i. 246; glycogen in, ii. 123; physiology, ii. 337, 338; respiration, ii. 42; resting cells, ii. 18
Ludwigii, ii. 5
lupuli, ii. 338*; description and properties, ii. 338, 339
pullulans, ii. 338,* 339
Oil, ii. 292, 334; in Dematium pullulans, ii. 283; in Mycoderma, ii. 305
Oil of mustard, action of, on yeast, ii. 181
Olease, i. 311
Oleate of potash. *See* Potassium oleate
Oleic acid, ii. 150, 206; action of air on, i. 152
Olive oil, i. 106, ii. 271
Ontjom, ii. 333
Oogonia, ii. 12
Oomycetes, motile spores, ii. 10; oogonia, ii. 10; relation to the Entomophtheæ, ii. 48; sporangia, ii. 11, 12; zoospores, ii. 12
Oospore, ii. 12
Ophidomonas, i. 27
jenensis, structure of cell contents, i. 33
sanguinea, morphology, i. 281*; physiology, i. 112*
Opium fermentation, ii. 271
Optical properties of cell wall, i. 30
Orange-flower water, mucinous decomposition, i. 219
Organic acids as antiseptics, i. 91
ferments, i. 17
stimulants of yeasts, ii. 180–182
Orleans process of vinegar-making, i. 306
Ornithin, ii. 412
Ornithopus, nodule bacteria, action on Vicia faba, i. 265
Orthodinitrokresol of potassium, i. 88
Oryza glutinosa, ii. 67, 333
Oscillaria, i. 23, 270
Osmic acid, ii. 106
Osmosis, ii. 44
Osteomyelitis, i. 73
Otomycosis, ii. 234, 238
Ovos, ii. 122, 344
Ox pancreas, ii. 118
Oxalate of ammonium. *See* Ammonium oxalate
of lime, ii. 86
of potassium. *See* Potassium oxalate
Oxalates, ii. 277
Oxalic acid, ii. 150, 262, 263, 264, 265, 266, 267, 276, 387, 390, 392, 395; action of, on yeasts, ii. 180; combination with lime, ii. 142; decomposition, ii. 42; excreted by fungi, ii. 44; from yeast gum by boiling with nitric acid, ii. 129; in Mucor Rouxii, ii. 65; produced by Aspergillus niger, ii. 259, 263; by A. phœnicis, ii. 239; by Sacch. Hansenii, ii. 171, 209
Oxalis, ii. 262, 264
Oxydases, i. 308–312, ii. 369; determination of, i. 308; isolation of, i. 310
Oxygen, i. 113, 114, 157, ii. 362, 363, 371, 372–375, 379, 387; action of, in liquefaction of gelatin, ii. 413, 414; in yeast fermentation, ii. 340; effect of amount of supply, on yeast respiration and reproduction, ii. 169–172; effect of, in fermentation by Allescheria Gayoni, ii. 272; influence on chemical activity of fungi, ii. 260, 265; on film-formation of Mycoderma, ii. 307; on form of Mycoderma cells, ii. 304; on Monilia sitophila, ii. 334; on Torulaceæ, ii. 295
Ozone as an antiseptic, i. 86; for purifying river-water, i. 86

Pachyma, cocos ii. 28
Pachyman, ii. 28
Paddy, ii. 63
Palmella prodigiosa. *See* Micrococcus prodigiosus
Palmitic acid, ii. 372; in yeast, ii. 127
Pancreas, i. 229, ii. 47, 118, 124
Pandalus borealis, ii. 302
Papilionaceæ, nitrogen-absorption from air by, i. 263
Paracasein, i. 184, 243
Paracholesterin, ii. 127
Parachromophorous, i. 105
Paradextran, ii. 29
Paraisodextran, ii. 29
Paralactic acid. *See* Lactic acid
Paranuclein, ii. 35, 118, 146
Parasites, facultative, i. 24; group of, ii. 74; obligate, i. 24
Parenchyma, ii. 6
Parietal layer, i. 33, 34
Paris butter, i. 153
Parthenospore, ii. 14
Parvoline, i. 233
Pasteur, fermentation theory of, i. 16, 19, 139, 143, ii. 340; rejects theory of spontaneous generation, i. 7, 16
Pasteur flask, i. 7, 77; Hansen's form of, ii. 161
Pasteur's nutrient fluid, i. 94

Pasteuria ramosa, forked branches, i. 265; longitudinal fission, i. 42
Pasteurisation, i. 161, ii. 103–105
Pastorianus type, ii. 84, 87
Paternoster pea, i. 234
Pathogenic bacteria, i. 73; nitrogenous nutriment of, i. 96; occurrence in butter, i. 160; and in milk, i. 155; species of Monilia, ii. 334
Pear must, ii. 158
Pectase, i. 169, ii. 271
Pectin, ii. 30, 108, 271; fermentation of, i. 152, 169, 170; ferments of, i. 152
Pectinase, ii. 270
Pectose, i. 151, ii. 29, 271
Pediococcus, definition of, i. 43
acidi lactici, division, i. 43; lactic acid fermentation, i. 172; morphology and temperature, i. 195
cerevisiæ, morphology, i. 221; sarcina-turbidity of beer, i. 221
Pe-fuh-ling, ii. 29
Peh-Khak, ii. 67
Pelargonic acid, ii. 372
Pemmican, i. 164
Penicillium, ii. 276, 303, 330, 403; action on cells, ii. 270; on fat, ii. 271; on oxalates, ii. 274; affinities, ii. 256; decomposing effects of, ii. 268; descriptive, ii. 219, 220, 221, 242, 243, 245, 246; behaviour with cadmium, ii. 33; lithium, ii. 32; and rubidium, ii. 31; colouring-matter, ii. 30; enzymes secreted by, ii. 271; iron not essential for, ii. 35; nucleus division, ii. 10; pathogeny, ii. 245, 246; proteolytic powers, ii. 273; sodium as food for, ii. 32
Penicillium album, ii. 249, 254
aromaticum, ii. 255, 256
aureum, ii. 244, 245, 255
bicolor, description, ii. 254
brevicaule, ii. 243,* 246, 252, 253,* 261, 275; alcohol produced by, ii. 272; behaviour with arsenic, ii. 38; liquefaction of gelatin by, ii. 274
Camembert, ii. 243,* 246, 248, 249, 254, 256
candidum, ii. 244, 248, 249, 254
cladosporioides, ii. 278; ammonia-producer, ii. 235
claviforme, ii. 243,* 244, 254
crustaceum, ii. 246; effect of light on, ii. 43
cupricum, ii. 256
descissens, ii. 255
Duclauxii, ii. 245, 246, 254
Epsteinii, ii. 249
geophilum, ii. 254, 256
glaucum, ii. 10, 153, 243,* 244, 245, 246, 247,* 248, 250, 251, 254, 256, 259, 260, 261, 262, 263, 264, 395; action of light on, ii. 43; action on acids, ii. 276; albumen, ii. 274, 275; action on arsenical substance, ii. 275; action on casein, ii. 274, 275; action on cells, ii. 270; action on eggs, ii. 281; action on fats, ii. 271; action on fibrin, ii. 274, 275; action on milk, ii. 274; action on peptone, ii. 274; action on sulphates, ii. 275; action on tannin, ii. 271; alcohol a food for, ii. 272; alcohol a source of carbon for, ii. 58; basipetal constriction, ii. 279; ammonia-producer, i. 235; behaviour towards cobalt, ii. 35; behaviour towards nickel, ii. 35; behaviour with arsenic, ii. 37; behaviour with zinc, ii. 33; description, ii. 220, 221; cæsium unsuitable for, ii. 31; casease secreted by, ii. 274; cell nucleus, ii. 10; cell wall, ii. 26; cellulose-dissolving enzyme, ii. 45; chemical composition, ii. 22; chemotropism, ii. 43; chitin content, ii. 28; cholesterin, ii. 127; conidia, ii. 15, 22, 32; decomposing effects, ii. 264, 268; development of mycelium, ii. 4*; diastase-formation, ii. 47; fission of glucosides by, ii. 269, 270, 271; and of polysaccharides, ii. 270; fructification, ii. 41; gelatin-liquefaction, ii. 46, 273; influence of lack of acid, ii. 58; inulase secreted by, ii. 270; lignification, ii. 30; lipase, ii. 47; magnesium an essential for, ii. 32; mechanical pressure, ii. 45; nitrogen-fixer, i. 271; pathogeny, ii. 275, 276; oxalic acid produced by, ii. 142, 262; pectinase secreted by, ii. 270; production of alcohol by, ii. 58; resistance to heat, ii. 22; respiration, ii. 42, 58; saccharification by, ii. 261
Penicillium granulatum, ii. 244, 254
humicola, ii. 255
insigne, ii. 244, 246, 255
italicum, ii. 220, 243,* 244, 245, 246, 250, 251,* 252; action on albumen, casein, fibrin, and milk, ii. 274, 275; decomposing effect of, ii. 269; fission of glucosides by, ii. 269, 270; inulase secreted by, ii. 270; saccharification by, ii. 261
luteum, ii. 243,* 244, 245, 246, 249, 250,* 252, 254, 255, 259; action on albumen, casein, fibrin, and milk, ii. 274, 275; cellulose - dissolving enzyme, ii. 46; decomposing effect of, ii. 269; description, ii. 220; fission of glucosides by, ii. 269; formation of acids by, ii. 265; gelatin-liquefier, ii. 46, 273; inulase secreted by, ii. 270; pathogeny, ii. 275, 276; pectinase secreted by, ii. 270; saccharification by, ii. 261
minimum, ii. 245
olivaceum, ii. 220, 243,* 244, 245, 252*; colouring-matter of, ii. 275; liquefaction of gelatin by, ii. 46, 273; pathogeny, ii. 275, 276

Penicillium pruriosum, ii. 245
purpurogenum, ii. 243,* 245; colouring-matter of, ii. 275; liquefaction of gelatin by, ii. 274
quadrifidum, ii. 245
radians, ii. 256
radiatum, ii. 245, 254
roseum, ii. 246, 254
Rogeri, ii. 249
Roquefort, ii. 246, 248, 256
rubrum, ii. 243,* 244, 245, 254; action on albumen, casein, fibrin, and milk, ii. 274, 275; colouring-matter of, ii. 275; decomposing effects of, ii. 269; fission of glucosides by, ii. 269, 270; inulase secreted by, ii. 270; liquefaction of gelatin by, ii. 273; saccharification by, ii. 261
silvaticum, ii. 255, 256
Wortmanni, ii. 244, 245, 255
Penicillopsis, description, ii. 219
Pentosan, ii. 29; in yeast, ii. 129
Pentosans of grain, ii. 151
Pentose from yeast nucleic acid, ii. 118
Pentoses, ii. 382; as source of carbon for yeast, ii. 150
Pepsin (peptase), i. 230, ii. 387, 392; action on albuminoids, ii. 118; action on casein, ii. 146; action on nuclein, ii. 118–126; discovery of, i. 17; from yeast, ii. 388; preparation from yeast culture, ii. 122
Peptone, ii. 377; action of endotryptase on, ii. 411, 413; as nutriment for peptone-carbon bacteria, i. 128; for phosphorescent bacteria, i. 124; for Torulaceæ, ii. 293; as source of nitrogen for yeast, ii. 155, 156; decomposition of, ii. 274; derived from casein, i. 231; in butter-milk, i. 251; in cheese, i. 243, 244; in Chlamydomucor oryzæ, ii. 68; influence on reproductive capacity of yeasts, ii. 166, 167; relation of, to frothy fermentation, ii. 134, 135; solution of, ii. 96
Peptones, ii. 345
Peptonised bouillon agar, i. 99
Peptonising enzymes produced by Bacillus anthracis, i. 230; B. fluorescens liquefaciens, i. 122; B. lactis erythrogenes, i. 108; B. liquefaciens lactis amari, i. 252; B. lupuliperda, i. 128; B. megaterium, i. 230; B. mesentericus fuscus, i. 135; B. mes. ruber, i. 135; B. mes. vulgatus, i. 134, 135; B. oogenes fluorescens α, i. 166; B. oogenes hydrosulfureus (α, β, γ, δ, ϵ, ζ), i. 166; B. prodigiosus, i. 106, 230; B. pyocyaneus, i. 230; B. saprogenes vini I.–VII., i. 239; B. subtilis, i. 103, 134, 230; by cholera bacillus, i. 103, 230; by Cladothrix dichotoma, i. 277; by the Kiel bacillus, i. 107; by Micrococcus acidi lactis, i. 172; M. ascoformis, i. 230; M. casei amari, i. 252; M. saprogenes vini I., II., i. 239; M. ureæ liquefaciens, i. 103; by Proteus mirabilis and vulgaris, i. 227; by Sarcina flava, i. 110; by Spirilla from cheese, i. 230; by Tyrothrix geniculatus, i. 252; by Vibrio Finkler-Prior, i. 230
Peridia, ii. 218 *et seq.*
Period of generation, i. 44, 45, ii. 164; influence of temperature on, ii. 165, 166
Perisporiaceæ, ii. 73; affinity, ii. 218
Perithecia, ii. 234, 241, 244, 255, 281; formation suppressed by light, ii. 41
Perithecial wall, composition of, ii. 28
Perithecium, ii. 73
Permanent cells, yeast, ii. 106; glycogen in, ii. 126
yeast, ii. 352, 353, 354, 355, 356
Permanganate solution, as test for arsenic, ii. 38
Permeability of cell membrane, ii. 167, 168
Peronocarpous, ii. 73
Peronospora viticola, ii. 173
Peronosporeæ, cellulose in, ii. 25; chitin in, ii. 28
Perry, ii. 328, 329
Petri dishes, i. 102
Petroleum spirit, i. 88
Peziza convexula, ii. 10
Fuckeliana, action of light on, ii. 40; heliotropism, ii. 39; oxalic acid as a metabolic product, ii. 142
Libertiana. *See* Sclerotium Libertiana
Phaseolus, nodule-formation, i. 265
vulgaris, ii. 239
Phenol, i. 227, ii. 390, 410; action on anthrax spores, i. 87; as an antiseptic, i. 87
Phenols, ii. 387
Phenylamidopropionic acid in Emmenthal cheese, i. 243
Phenylhydrazine, ii. 394
Phenyl-salicylate, ii. 271
Philothion, ii. 147, 415, 416
Phloretin, ii. 269
Phloridzin, ii. 269
Phloroglucin, action of, on yeasts, ii. 181
Phloxin red in typhus bacillus, i. 34
Pholiota squarrosa, ii. 256
Phosphate of ammonia. *See* Ammonium phosphate
Phosphates, ii. 348, 349, 351; action of, on proteolysis, ii. 411
Phosphocarnic acid, ii. 146
Phosphomolybdate of ammonia, ii. 37
Phosphorescence, i. 123 *et seq.*
Phosphoric acid, ii. 275, 367; decomposition of paranuclein, ii. 118; in fungi, ii. 31; in plant ash, ii. 31; in yeast gum, ii. 128; in yeast water, ii. 412; meta-, in the yeast cell, ii. 146; reaction with yeast ash, ii. 140; requirements of the yeast cell for, ii. 143
Photobacteria, use of, in testing efficiency of filters, i. 78
Photobacterium, activity of, i. 123, 124; as test for enzymes, i. 125; culture of, i. 126; foodstuffs for, i. 126; infection of small marine animals, i. 126; requisite food for, i. 124; spectrum, i. 126

Photobacterium balticum, i. 124
Fischeri, i. 124
indicum, i. 124
javanense, i. 124
luminosum, i. 124
Pflügeri, i. 124, 125
phosphorescens, i. 123, 124, 125
sarcophilum, effect of light on luminosity of, i. 62, 63
Phototropism, ii. 39
Phragmidiothrix, i. 272, 275
multiseptata, i. 275
Phycomyces, development of mycelium, ii. 4, 5; rheotropism, ii. 44; suspensors, ii. 50
nitens, chemotropism, ii. 43; heliotropism, ii. 40; hydrotropism, ii. 13; influence of Röntgen rays on, ii. 43; occurrence, ii. 50; oxalic acid produced by, ii. 142; respiration, ii. 42; sporangiophores, ii. 56
Phycomycetes, ii. 3, 14, 18
Phyllanthus emblica, ii. 240
Physiological salt solution, i. 33
Phytosterin, ii. 127
Pichia, ii. 303; definition, ii. 202, 211, 212; sporulation experiments with, ii. 192
californica, description, ii. 212
farinosa, description, ii. 213
membranæfaciens I., description, ii. 212; II. and III., description, ii. 212
Radaisii, description, ii. 213
tamarindorum, description, ii. 213
taurica, description, ii. 212, 213
Pickles as a culture medium, ii. 232
Picric acid, ii. 109, 115
Piloboleæ, sporangia, ii. 50
Pilobolus, heliotropism, ii. 39
cristallinus, action in blue light, ii. 39; behaviour in the ultra-red rays, ii. 40; occurrence, ii. 50
microsporus, after-effect of illumination, ii. 41; behaviour in the yellow light, ii. 39
Pinguicula alpina and vulgaris, i. 186; for production of thick milk, i. 216; proteolytic enzyme, i. 231
Pinhead bacteria, i. 47
Pink yeasts, ii. 288, 299, 300
Piqûre, ii. 238
Pitching yeasts, i. 188
Plant juices, discoloration of fresh, i. 309
Plantago psyllium, ii. 29
Plants as a source of mineral matter, i. 36; carnivorous, i. 231; diseases of, i. 214
Plasmic acid, ii. 118; iron content of, ii. 35
Plasmic theory of fermentation, ii. 357
Plasmodial cords, i. 267
Plasmolysis, i. 32, 46
Plastin, ii. 121
Plate cultures, ii. 99, 101; of anaerobes, i. 142; of luminous bacteria, i. 125; with agars, i. 101
Plate pouring apparatus, i. 101
Platinum black, action on vinegar fermentation, i. 14, 304
Plectenchyma, ii. 337
Pleochroism in stained bacteria, i. 31
Pleomorph, ii. 19; in yeast, ii. 76
Pleomorphism, i. 28, 70, 71, 72, ii. 19, 84, 85
Pleospora herbarium, ii. 279
Plums, ii. 324, 331
Pneumobacillus, membrane, i. 31; mutability, i. 72
Pneumomycosis, ii. 234
Podocarpus, nodule-formation in, i. 263, 264
Polar flagella, use of, in study of bacteria, i. 39
Pollinodium, ii. 246
Polyporea, ii. 37
Polyporus officinalis, cellulose in, ii. 25; fongose in, ii. 29
Polysaccharides, ii. 381; fission of, by Aspergillaceæ, ii. 270
Polysaccharomyces, ii. 151
Pombe, ii. 216
Populin, ii. 45; fission of, ii. 269
Potash, ii. 365; bath, i. 120; solution, ii. 32
Potassium as food for bacteria, i. 56; as inhibitor of movement, i. 32; bichromate, as a milk preservative, i. 161; bitartrate, ii. 145; carbonate, behaviour of yeast to, ii. 139; chlorate, ii. 108; chloride, behaviour of Eumycetes to, ii. 32; nitrate, i. 109, ii. 32; permanganate, ii. 179; phosphate, behaviour of yeasts to, ii. 139, 146; phosphates, i. 95, 122; sulphate, ii. 32, 139
Potassium acetate, ii. 150
alum, action of, on yeasts, ii. 179
arsenite, action of, on yeast, ii. 174, 179
carbonate, ii. 348
chloride, ii. 410
citrate, ii. 150
dextro-tartrate, ii. 150
formate, ii. 150
glycerate, ii. 150
lactate, ii. 150, 365
malate, ii. 150
nitrate, ii. 403
oleate, ii. 150, 206
oxalate, ii. 150
permanganate, i. 161; action of, on yeast, ii. 179
phosphate, ii. 311; action of, on yeast, ii. 405
propionate, ii. 150
Potato bacillus, i. 80, 134, 135,* 235
culture, i. 101, 103
glass, ii. 38
juice as an attraction for bacteria, i. 41
Preservation of cultures with formalin, i. 103
Preserved meat, i. 163
Pressed yeast Nos. 430, 487, 574, ii. 393; Winterhude Race III. No. 139, ii. 393
Pressed yeast cell, influence of light on, ii. 40, 41

Pressed yeast manufacture, flocculence in, ii. 93
Primary generation, i. 3
Promycelia, ii. 300
Propionate of potassium. *See* Potassium propionate
Propionic acid, ii. 150, 276, 372
Propyl alcohol, i. 140, ii. 150, 375, 378; action of, on grape sugar, ii. 178
Prosthetic side chain, ii. 118
Protagon, ii. 383
Protalbuminose, ii. 349
Protease, ii. 274
Proteid materials, ii. 303
Proteids, ii. 334, 347, 349, 350, 351, 355, 370; action of Aspergillaceæ on, ii. 273–277; decomposition of, by yeast, ii. 408
Protein, ii. 263, 340, 344, 369, 371, 372, 378; action of endotryptase on, ii. 415; as foodstuff, ii. 334; derived from asparagin by yeast, ii. 155; in rye, ii. 155
Proteolysis, ii. 408 *et seq.*, 411, 412, 413; in asporogenic cells, ii. 191
Proteolytic enzymes, i. 230–232; in Eumycetes, ii. 46; testing for, with thymol-gelatin, i. 230, 231
Proteoses, ii. 345
Proteus, i. 69
  mirabilis, locomotion, i. 227; morphology and involution forms, i. 227; peptonising enzyme, i. 227
  sulfureus, i. 228
  vulgaris, ammonia-producer, i. 235; invertin, i. 213; involution forms, i. 227; locomotion, i. 227; morphology, i. 227*; nutrient for, i. 227; peptonising enzyme, i. 227; urea decomposed by, i. 228
  Zenkeri, i. 227, 228
Protomyces, ii. 312; spores, ii. 79
Pseudo-acetic acid, i. 139
Pseudo-Dematophora, action on cellulose, ii. 45
Pseudo-globulin, ii. 411
Pseudo-nucleins, ii. 118
Pseudo-parenchyma, ii. 6
Pseudopodia, i. 40
Pteridophyta, i. 22
Ptomaines, i. 232–234
Puccinia graminis, æcidia, ii. 17
  suaveolens, ii. 288
Punceria coagulans, lab in, i. 186
Puncture culture, i. 103
Pure cultures, i. 66, 96, 103, 132, 246, 247, ii. 159–164, 304
Purification (self-) of rivers, i. 61, 62
Purin bases, ii. 119, 120
Purple bacteria, assimilation in the dark, i. 114; assimilation and oxygen-elimination, i. 113, 114; fission, i. 282; influence of spectrum colours on, i. 113; morphology, i. 111; resistance to light, i. 63
Pus cells, nuclei, ii. 117
Putrefaction, distinction between fermentation and, i. 6, 223; Stahl's theory of, i. 10; yeast, i. 232
Putrefactive bacteria, activity in natural wine must, i. 67; development in the cadaver, i. 163, 164; effect of organic acids on, i. 91; guanin changed by, ii. 119; mycoprotein in, i. 35; occurrence in the alimentary canal, i. 228; species of, i. 226–230
Putrescine, i. 233
Pycnides, ii. 17
Pyocyanine, i. 121
Pyrazin derivatives, ii. 380
Pyrenomycetes, affinity, ii. 218; asci, ii. 29; perithecium, ii. 73
Pyridin, ii. 380, 413
Pyrimidin, methyl-dioxy-, ii. 119
Pyrocatechin, action of, on yeast, ii. 181
Pyrogallic acid, i. 14, 141
Pyrogallol, action of, on yeast, ii. 181
  tube, Buchner's, i. 141,* 142
Pyrotartaric acid, decomposition by, from fungi, i. 240
Pythium anguillulæ aceti, i. 307

Quargel cheese, i. 148
Quark, i. 184, 243
Quercitol, ii. 150
Quercitrin, ii. 269
Quinine sulphate, ii. 349

Rabbits, ii. 102
Race II., a yeast designation, ii. 81; action of sunlight on, ii. 42; Amylomyces process, ii. 70; froth fermentation, ii. 134
Racemic acid, i. 176, 177
  compounds, fission of, i. 177–178
Racking of young beer, ii. 135, 136
Raffinase, ii. 397–399; secreted by Aspergillus niger, ii. 268; by Penicillium glaucum, ii. 268
Raffinomyces, ii. 151
Raffinose, ii. 15, 150, 208, 211, 296, 391, 397, 398, 406; action of β-Amylomyces, ii. 66; on Mucor Rouxii, ii. 65; on Mucors, ii. 61; behaviour of luminous bacteria towards, i. 125; fermentation of, ii. 332, 335; ferments of, ii. 215, 216, 217; fission of, 268
Ragi, ii. 56, 67, 68, 206, 333
Raisins as food for Schizosacch. octosporus, ii. 155
Rancidity of fats, i. 152, 153
Ranvier's chamber, ii. 101
Raspberries, ii. 324
Raulin's nutrient solution, ii. 16, 17, 41, 57, 294
Red grains, i. 33*
  wines, warming, ii. 104
  yeasts, ii. 299–302; nutrient material for, ii. 301
Reddening of cheese, i. 109; of milk, i. 108–109; stockfish, i. 109
Reductases, ii. 415
Rennet, formation by Bacillus lactis erythrogenes, i. 108; liquefaciens lactis amari, i. 252; mesentericus vulgatus, i. 135; Bacterium coli commune, i. 186; B. synxanthum, i. 109; Micrococcus prodigiosum, i. 186; Sarcina rosea, i. 108;

Tyrothrix catenula, T. claviformis, T. distortus, T. filiformis, T. geniculatus, T. scaber, T. turgidus, T. urocephalus, i. 245; coagulation by, i. 184; powder, solution, tabloids, i. 186
Reproduction of yeast cells, ii. 164–172; action of alcohol on, ii. 175
Reproductive capacity, ii. 164
energy or power of bacteria, i. 44, ii. 164, 165, 168
Repulsion, ii. 43
Resin reaction, ii. 86
Resorcin, action of, on yeasts, ii. 181
Respiration in Torula, ii. 301; in yeasts, ii. 170–172
Respiration figures, i. 41
Retting, ii. 238; mixed, i. 151, 152
Revertobiose, ii. 391
Revertose, ii. 391
Rhabdochromatium, i. 282
Rhabdomonas rosea, i. 112,* 281*
Rhamnose, ii. 382; as a glycogen-former, ii. 125
Rheotropism, ii. 44
Rheum, ii. 265
Rhizoid processes, in Mucor Cambodja, ii. 67
Rhizoids, ii. 55
Rhizomorpha, ii. 39
Rhizopus nigricans, ii. 22, 262; action of arsenic, ii. 37; barium, ii. 34; beryllium, ii. 33; cadmium, ii. 33; cæsium, ii. 31; calcium, ii. 34; glucose, ii. 43; lithium, ii. 32; maltose, ii. 61; potash, ii. 32; rubidium, ii. 31; saccharose, ii. 43, 61; selenium, ii. 36; sodium, ii. 32; strontium, ii. 34; zinc, ii. 33; alcohol-formation, ii. 60; cellulose-dissolving enzyme, ii. 45; chemotropism, ii. 43; chitin content, ii. 28; effect of light on, ii. 40; germination, ii. 40; germs with hyphæ, ii. 45*; heliotropism, ii. 39; intramolecular respiration, ii. 58; oxalic acid as a metabolic product, ii. 142; sporangia, ii. 41; stolon, ii. 55,* 56; succinic acid formation, ii. 61
Rhizopus oryzæ, in ragi, ii. 67; sporangia, ii. 56
ramosus. *See* Mucor ramosus
stolonifer, sporangiophore, ii. 13
Rhodomyces Kochii, action of light on, ii. 40
Rhodonate compounds in cheese, i. 109
Rhus cotinus, i. 309
vernicifera, i. 309
Rice, ii. 270; action of Aspergillus oryzæ on, ii. 260; Japanese and Tonquinese, ii. 65
beer, ii. 102
Rich cheese, i. 184
Ricin, i. 234
Ricinus communis, i. 234
Rivers, self-purification of, i. 61, 62
Roberts' boiling method, i. 30, 53, 131, 132
Robinia, nodule-formation of, i. 265
Roll cultures, i. 102, 141
Röntgen rays, effect on bacteria, i. 61; on Eumycetes, ii. 43
Ropy milk, i. 215; whey, i. 216; wine, i. 217
Roquefort cheese, ii. 248, 256, 274
Rousing, ii. 373, 374
Rubber cap, i. 76
Rubia tinctorum, i. 108
Rubin, *α* and *β* oryzæ, ii. 11
Russula nigricans, oxydising enzyme, i. 311
Rust fungi. *See* Uredineæ
Rye, pentosans in, ii. 151; protein in, ii. 155

Saaz yeast, ii. 203, 393, 397, 402; action of acetic acid on, ii. 180; bottom fermentation, ii. 81; influence of asparagin on reproductive capacity, ii. 166, 167; influence of carbon dioxide on, ii. 178; influence of temperature on period of generation, ii. 165
Saccharin, action of, on yeasts, ii. 182
Saccharobacillus pastorianus, chemical activity, i. 194; effect of temperature, i. 195; food for, i. 195; in white beers, i. 196
Saccharomyces, i. 13, ii. 199 *et seq.*, ii. 286, 289, 332; asporogenation in, ii. 193; definition of, ii. 202; derivation of, from Aspergillus, ii. 80; philothion in, ii. 416; phosphorus content of, ii. 37; species of, ii. 202–209
Saccharomyces acetethylicus, ii. 150, 154
acidi lactici, ferments lactose, ii. 208
albicans, ii. 334
anomalus, ii. 202, 213, 307; ascospore, ii. 74, 97*; granules, ii. 113; influence of temperature on period of generation, ii. 165; limits of temperature, ii. 165; var. I., ii. 214; vars. II. and III., ii. 214; var. belgicus, ii. 215
apiculatus, ii. 111, 183, 290, 370, 382, 383, 397, 405; description, ii. 199, 314; effect of bouillie Bordelaise on, ii. 173; effect of sunlight, ii. 42; effect of, on activity of wine yeasts, ii. 320–329; effect of Sacch. ellipsoideus on, ii. 325; fermentation by, ii. 324–329; form of cells, ii. 314, 315,* 316, 317; granules, ii. 113; growth and nutrition, ii. 318–320; habitat and breeding-place, ii. 183, 184, 187; long duration of, ii. 320; mixed cultures with Sacch. cerevisiæ, ii. 318; morphology and characters, ii. 314–329; nutrient media for, ii. 319; occurrence in fruits, ii. 324–329; production of oxydases, i. 309; sensitiveness to chemical and physical agents, ii. 319, 320; sulphuretted hydrogen, ii. 147; variation, ii. 316, 317
aquifolii, description, ii. 206
awamori, action of alcohol on, ii. 175, 176
Bailii, ii. 382, 383; description, ii. 207; effect of strong nutrient media on, ii. 167
Bayanus, description, ii. 206
capsularis, ii. 201 ["rius" is a misprint]
cerevisiæ, i. 13, ii. 30, 199, 200, 289, 332, 346, 385, 386, 389, 401; asporogenation, ii. 191; cellulose not detected

in, ii. 26; description, ii. 202, 203; habitat, ii. 184; lignification, ii. 30; mixed culture with Sacch. apiculatus, ii. 318; variety of, ii. 196
Saccharomyces cerevisiæ I., ii. 97,* 152, 159, 377; action of tartaric acid on, ii. 180; ascospore-formation, ii. 99; cells from sedimental yeast, ii. 82, 83*; duration of film, ii. 88; granules, ii. 112; influence of carbon dioxide on, ii. 178; limits of temperature, ii. 165; name changed to S. cerevisiæ, ii. 201, 203; production of zymase by, ii. 191
conglomeratus, ii. 199
cratericus, ii. 397, 402
ellipsoideus, ii. 199, 200, 317, 318, 402; description, ii. 205; effect of sunlight, ii. 42; habitat, ii. 184, 185, 186; influence on Sacch. apiculatus, ii. 325; occurrence on grapes, ii. 325; production of oxydases, i. 309
ellipsoideus I., ii. 153, 159, 205, 383, 386; asporogenation in, ii. 191; cells, ii. 83*; film cells, ii. 89, 90*; granules, influence of carbon dioxide on, ii. 178; influence of temperature on period of generation, ii. 165; limits of temperature, ii. 165
ellipsoideus II., ii. 159, 205, 383, 397; beer turbidity, ii. 98; film cells, ii. 89; granules, ii. 112; influence of carbon dioxide on, ii. 178; influence of temperature on period of generation, ii. 165; limit of temperature, ii. 165; resisting power of spores, ii. 102; top-fermentation habit acquired by, ii. 194, 195
ellipticus, ii. 399
exiguus, ii. 199, 382, 405; description, ii. 207
farciminosus, ii. 81
farinosus, ii. 213, 382, 383; effect of strong nutrient media on, ii. 167
flava lactis, ii. 208, 299
fragilis, description, ii. 209
fragrans, ester of, ii. 150
galactocola, ii. 289
glutinis, ii. 299; granules, ii. 112
guttulatus, ii. 211; sporulation, ii. 102, 103*; vacuoles, ii. 110
Hansenii, ii. 370; description, ii. 209; metabolic products of, ii. 171
hyalosporus, ii. 200
ilicis, description, ii. 206
intermedius, asporogenation, ii. 191; description, ii. 204; variability of, ii. 189
japonicus, ii. 300; action of alcohol on, ii. 176
Jörgensenii, description, ii. 207, 208
Jordermanii, description, ii. 206
Kefyr, ii. 150, 289, 292, 293, 395; giant cells, ii. 85; lactase of, favouring luminosity of bacteria, i. 125
Keiskeana, ii. 300; action of alcohol on, ii. 176
Saccharomyces lactis, ii. 208, 289, 290, 292, 294, 295, 296, 297
lactis acidi, ii. 208
lithogenes, ii. 81
Ludwigii, ii. 163, 201, 210; budding, ii. 111*; cell-fusion, ii. 101; fat-colouring, ii. 127; influence of temperature on period of generation, ii. 165; karyokinesis, ii. 110; limits of temperature, ii. 165; spore-germination, ii. 20, 100, 101; spores in fluid culture, ii. 96; vacuoles, ii. 110
mali, description, ii. 206, 208
Marxianus, ii. 383; description, ii. 206, 207; effect of dextrin on spore-producing power, ii. 191; limits of temperature, ii. 165
mellacei, ii. 217
membranæfaciens, ii. 202, 382, 383; alcohol-fermentation, ii. 78; film-formation, ii. 90; influence of temperature on period of generation, ii. 165; limits of temperature, ii. 165; spores, ii. 96
membranæfaciens II., ii. 212
membranæfaciens III., ii. 212
membranæfaciens, var. californica, ii. 212
membranæfaciens, var. tamarindorum, ii. 213
(Meyen), a generic name for yeast, i. 13
mycoderma, ii. 199, 200; effect of agitation on, i. 63
neoformans, ii. 81
niger, ii. 302; fermentative action, ii. 302
orientalis, asporogenation in, ii. 191
ovalis, ii. 81
Pastorianus, ii. 147, 184, 199, 200, 399; asporogenation, ii. 191; cells, ii. 84, 85*; description, ii. 204
Pastorianus I., ii. 159, 383, 393, 397; action of tartaric acid on, ii. 180; cell nucleus, ii. 109; in bitter beer, ii. 98; influence of sunlight, ii. 42; influence of temperature on period of generation, ii. 165; limits of temperature, ii. 165; name changed to Sacch. Pastorianus, ii. 201, 204; sporulation, ii. 95; sporulation experiments with, ii. 192–194
Pastorianus II., ii. 159, 383, 391, 397, 403; cell nucleus, ii. 109; film cells, ii. 89; influence of carbon dioxide on, ii. 178; influence of temperature on period of generation, ii. 165; limits of temperature, ii. 165
Pastorianus III., ii. 159, 383, 393, 397, 403; action of tartaric acid on, ii. 180; beer turbidity, ii. 89, 98; film cells, ii. 88,* 89*; influence of carbon dioxide on, ii. 178; influence of temperature on period of generation, ii. 165; modification of habit in, ii. 195; mortal temperature, ii. 104; name changed to Sacch. validus, ii. 201, 204

Saccharomyces pinophthorus melodus, ii. 296, 297
productivus, i. 383
pyriformis, description, ii. 206; chain of buds, ii. 8*; effect of sunlight, ii. 43; symbiosis with Bac. vermiformis, i. 66, 196*; in ginger-beer, i. 196
Radaisii, ii. 213
Rouxii, description, ii. 208
ruber, ii. 299
Saké, description, ii. 206
saturnus, ii. 215
Soja, description, ii. 208
sphæricus, ii. 81
theobromæ, ii. 163
turbidans, description, ii. 205
tyrocola, ii. 289, 290, 292, 295, 296, 297, 395
validus, ii. 201; asporogenation, ii. 191; description, ii. 204, 205; found in beer, ii. 205
vini, i. 13, ii. 200
Willianus, ii. 205
Zopfii, ii. 150, 154, 207; effect of strong saccharine solutions on, ii. 167; resisting power, ii. 103
Saccharomycetaceæ, classification, ii. 199-217; analysis of genera, ii. 202
Saccharomycetes, ii. 8, 10, 287, 289, 290, 291, 292, 293, 294, 298, 299, 300, 301; definition, ii. 202; ascus, ii. 72; chitin absent in, ii. 28; life-history, &c., ii. 183–217; position in plant kingdom, ii. 72, 73–76 *et seq.*; variability of, ii. 189–198
Saccharomycodes, ii. 201, 203; sporulation experiments with, ii. 192
Behrensianus, description, ii. 210, 211
Ludwigii, asporogenation in, ii. 191; description, ii. 210; sporing power restored, ii. 191
Saccharomycopsis, definition, ii. 201, 203; description, ii. 211
capsularis, description, ii. 211
guttulatus, ii. 165; description, ii. 211
Saccharomycosis, ii. 82
Saccharose, ii. 15, 134, 150, 151, 163, 283, 302, 345, 346, 360, 377, 383, 385, 386, 388, 398, 406, 413; action of, on proteolysis, ii. 410, 411; action of Sacch. Hansenii on, ii. 171, 209; action of zymin on, ii. 353; action on living organisms, ii. 370; as a culture medium, ii. 190; as a stimulant, ii. 44; as glycogen-former, ii. 125; as nutriment for Torulaceæ, ii. 293; attacked by Mycoderma, ii. 311, 312; not by Saccharomyces apiculatus, ii. 319, 321; behaviour with β-Amylomyces, ii. 66; with Mucor Rouxii, ii. 66; with Rhizopus nigricans, ii. 44; decomposition of, by species of Mucor, ii. 61; diastase-formation, ii. 47; fermentation of, ii. 331, 332, 337, 342, 365, 367, 368, 380; by Bac. amylozyme, i. 147; by Saccharomyces apiculatus when mixed with glucose, ii. 321; by Torulaceæ, ii. 293, 301; ferments of, ii. 202, 206, 210, 214, 215, 216; non-ferments of, ii. 208, 210, 211, 214, 217; influence on reproductive capacity of yeasts, ii. 167; inversion of, ii. 239, 260, 268; inversion by Bacillus subtilis, i. 134
Sachsia albicans, description and properties, ii. 335*
suaveolens, ii. 333, 403; description and properties, ii. 335*
Sacks, disinfecting dirty, i. 85, 86
Safranin, ii. 121
Sage cheese, i. 246
Saké, ii. 103, 206; yeast, ii. 176, 177, 228; action of tartaric acid on, ii. 180
Salicin, ii. 45, 150; fission of, ii. 269
Salicylate of ammonia. *See* Ammonium salicylate
Salicylic acid, i. 88, 161, 219, 222, 270, ii. 387, 410; action of, on yeasts, ii. 181
aldehyde, ii. 269
Saligenin, ii. 269
Salt as a preventive of puffiness in cheese, i. 251; effect on enzymes, ii. 260, 269
solution, physiological, i. 33
Salting of meat, i. 164, 165
Saltpetre-formation, i. 293
Sand-filter, i. 77
Sandy soil, germ content, i. 138
Sapocarbol, i. 88
Saponin, ii. 270
Saprine, i. 233
Saprol, i. 88
Saproligniеæ, cellulose in, ii. 25; chitin absent in, ii. 28
Saprophytes, i. 23, ii. 74
Sarcina, definition, i. 43; as cause of slimy white beer, ii. 133; beer sickness, i. 221; turbidity of beer, i. 221; with red and yellow pigments, i. 110
Sarcina aurantiaca, colouring-matter, i. 110
flava, action on fumaric acid, i. 219; peptonising enzyme, i. 110
lutea, ammonia-producer, i. 235; colouring-matter, i. 110
maxima, division in three directions, i. 43; morphology, i. 195
mobilis, locomotion, i. 39
rosea, action of light on, i. 62; coloration of milk, i. 108
ventriculi, i. 26,* 43,* 110*
Sarcolactic acid. *See* Lactic acid
Sarkin, ii. 121, 412
Sauerkraut fermentation, i. 203
Sausage poisoning, i. 234
Scarlet fever, milk as a carrier, i. 156
Scenedesmus acutus, purification by plate culture, i. 103
Sceptromyces opizii, ii. 237
Schinzia leguminosarum, i. 267
Schizomycetes, i. 22, 23, 69, ii. 1, 3
Schizophytæ, i. 22, 23
Schizosaccharomyces, ii. 163; cell-multiplication, ii. 75, 76; description, ii. 216
mellacei, ii. 382, 403; description, ii. 217; production of mycelium in, ii. 194
octosporus, ii. 389, 394, 403; asporogenation in, ii. 191; description, ii. 194;

karyokinesis, ii. 110; membrane, ii. 107; multiplication, ii. 75; nitrogen sources for, ii. 155; spores, ii. 29; sporulation, ii. 97; sugar as food for, ii. 150; vacuoles, ii. 110
Schizosaccharomyces Pombe, ii. 382, 383, 397, 402, 403; description, ii. 216; production of mycelium, ii. 194; production of zymase by, ii. 191; vacuoles, ii. 110
Schizosaccharomycetaceæ, affinities, ii. 199; classification, ii. 216, 217
Schulze's reagent, ii. 26, 108
Sclerotia, ii. 6, 220, 239, 240, 244, 245, 248, 251, 253, 254, 255
Sclerotinia cinerea, ii. 334
fructigena, ii. 334
Fuckeliana, cellulose-dissolving enzyme, ii. 45; conidia, ii. 73; pleomorphism, ii. 77
sclerotiorum, ii. 262
Sea-water as nutrient medium for luminous bacteria, i. 125
Segmentation, i. 44
Selective process, i. 36
Selenium, ii. 36
Seminose, ii. 382
Senega, ropy infusion of, i. 218
Sepsin sulphate, i. 232
Sepsis, i. 232
Septa, ii. 3, 7, 11
Septation, ii. 5
Septicæmia, i. 232
Serum therapeutics, i. 235
Sewage, purification of, by electricity, i. 57
Shojou, i. 248
Silica in yeast, ii. 141, 144
medium, i. 101, 138
Silicon, ii. 36
Silkworm disease, ii. 233
lethargy, i. 55
Silo, i. 202
Silver nitrate, ii. 387, 390, 392, 416
Single-cell culture, ii. 159–164
Siris, ii. 344
Skatole, i. 224
acetic acid, i. 224
Skim cheese, i. 184
Small-drop culture, ii. 99, 162
Small intestines, bacteria in, i. 163, 229
Smoked meat, i. 165
Smut fungi. *See* Ustilagineæ
Smut in oats, ii. 79
Snake poison, i. 234
Snuff, i. 129
Société d'Amylo, ii. 69
Soda, ii. 390
Sodium acetate, ii. 150, 179
arsenite, ii. 349; action of, on yeast, ii. 174, 179
azoimide, ii. 349
borate, ii. 411
butyrate, ii. 150
carbonate, ii. 390, 392
chloride, i. 124; ii. 349, 405, 410
fluoride, ii. 349
formate, i. 143
Sodium hydroxide, ii. 387, 392
indigo sulphate, i. 143
lactate, ii. 150
nitrate, i. 67, 68
nitrite, ii. 349
sulphate, ii. 349
Soil as a habitat of Saccharomycetes, ii. 183–185; bacterial content of, i. 137
Soja, ii. 213
Soja-bean, ii. 230, 270; fermentation, i. 247; nodule bacteria in, i. 265
Solanin, ii. 269
Solid colonies, i. 103
Solutol, i. 88
Solveol, i. 88
Sorb apples, ii. 324
Sorbinose, effect on sporangia-formation, ii. 15
Sorbite, influence on sporangia-formation, ii. 15; oxidation to sorbose, i. 305
Sorbose, derivation from sorbitol, i. 305; glycogen-former, ii. 125
Sorbus aucuparia, i. 305
intermedia, i. 305
latifolia, i. 305
Soxhlet bottle, i. 157
Soy (Shojou), i. 248
Spalen cheese, i. 243
Sparkling fermentation, ii. 137
Sphærella, ii. 278
intermixta, ii. 73
Tulasnei, ii. 73
Sphæria carpophila, growth to light, ii. 40
Sphæriaceæ, ii. 73
Sphærobacteria, i. 69
Sphærococcus lactis acidi, i. 172
Sphærotilus natans, i. 274
roseus, i. 274
Sphærulina, ii. 278, 281
intermixta, ii. 281, 282*
Spherical yeast, ii. 7
Spiny arthrospores, i. 51
Spirillum, i. 26, 27, 69
desulfuricans, action on gypsum, i. 225, 226; artificial culture, i. 48
endoparagogicum, spores, i. 46, 47*; spore-development, i. 54, 55
luteum, artificial culture, i. 48; colouring-matter, i. 48; linen as a source for nitrogen, i. 271
marinum, artificial culture, i. 48
rubrum, artificial culture, i. 48; morphology, i. 281; physiology, i. 112
tenue, i. 113
tyrogenum, fission of fat, i. 153; formation of dextrolactic acid, i. 179
undula, i. 39, 113
volutans, i. 25,* 112,* 281*
Spirit of wine produced by species of Mucor, ii. 60
Spiritus vini, ii. 359
vitis, ii. 359
Spirobacillus Cienkowski, mutability, i. 72
Spirobacteria, i. 70
Spirochæta, i. 25, 69, 70
Denecke, i. 25*

Spirochæta, Koch's cholera organism, i. 25*
Obermeieri, i. 25*
of human dental mucus, i. 25*
serpens, i. 33
Spirogyra, ii. 34
Splitting effect by Bacillus typhi abdominalis, i. 153; by B. pyocyaneus, i. 153; Denecke's Spirillum, i. 153; Micrococcus tetrasporus, i. 153; Vibrio choleræ Asiaticæ, i. 153
Spontaneous combustion of hay and cotton, i. 127
fermentation of fruit, i. 19, ii. 51, 58; of yeast, ii. 125
generation, i. 3, 4, 5, 8, 9
heating of bark, cotton, hay, i. 127; and hops, i. 128
Sporangia, distribution, ii. 12; formation, ii. 12; fructification, ii. 11, 12, 49; influence of moisture on formation, ii. 14, 15
Sporangioles, ii. 49
Sporangium, ii. 11*
Spore plasma, water content of, i. 50
Spores, i. 7, 22, ii. 246, 248, 250, 255, 314, 315; as characteristics, ii. 200, 201; ascospores, ii. 94–99; classification, ii. 14; conidiophores, ii. 15; effect of heat, ii. 22; form, i. 47; formation of, first observed by Perty, i. 55; germination, i. 52, ii. 3, 20, 40, 99; influence of temperature, ii. 95; mycelium-formation, ii. 2; of Ascoidea rubescens, ii. 75; resistance to drying up, ii. 21, 22; size, i. 49, ii. 96, 97; tenacity of life, ii. 21; time-limit of sporulation, ii. 97, 98
Sporodinia aspergillus, ii. 20*
grandis, fructification, ii. 14; in snuff-fermentation, ii. 62; sporangiophores, ii. 13; zygospores, ii. 14, 15
Sporogenic granules, i. 33, 34, 46
Sporonema gracile, i. 55*
Sporotrichum, ii. 281
Sporulation, effects of temperature on, ii. 191, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215; experiments on yeasts, ii. 192 *et seq.*
Sputum, i. 51
Stachyose, ii. 383, 399
Stahl's fermentation theory, i. 10
Stain for nucleic acid, ii. 121
Staphylococcus, i. 26,* 42
albus, cholesterin, ii. 127
pyogenes albus, effect of sunlight, i. 62
pyogenes aureus, action of formalin, i. 90; action (pathogenic, chromogenic, and zymogenic), i. 73; colouring-matter, i. 108, 110; effect of sunlight on, i. 62
Starch, ii. 334, 336, 376, 380, 388, 389, 399; action of Aspergillus oryzæ on, ii. 270; as nutrient material, ii. 301; conversion of, to dextrin, by Bacillus suaveolens, i. 147; decomposition of, by Bacillus œdematis maligni, i. 140; fermentation of, ii. 61; glucose formed from, i. 147; saccharification, i. 147, ii. 235, 239, 260–261; by species of Mucor, ii. 61
Starch, animal, ii. 123
sugar, ii. 382
Steam steriliser, Gaffky, i. 81; Koch's, i. 81
Steapsin, ii. 47
Stearic acid, ii. 150, 206, 372; in yeast, ii. 127
Steinberg yeast, ii. 326–328
Stereoisomer, i. 175
Sterigmata, ii. 219 *et seq.*, 227, 228,* 229,* 230, 231,* 232, 233,* 234, 235, 236,* 237, 238,* 239, 240, 241,* 242, 243, 248, 249, 250,* 251, 252, 253, 254, 255, 256, 257, 330
Sterigmatocystis, description, ii. 219, 220, 221
antacustica, ii. 237
ficuum, ii. 239
nidulans, ii. 220, 240
niger, ii. 237
pseudonigra, ii. 240
pulverulenta, description, ii. 239
sp., ii. 240
versicolor, ii. 242
Sterilisation by direct steam, i. 81; by dry heat, i. 80; by moist heat, i. 81, 82; by pressure steam, i. 82; mixed, i. 92; of albuminoids by cold, i. 89; of glass, i. 80, 84; of milk by Neuhauss, Grönwald, and Öhlmann's method, i. 159; by Soxhlet's method, i. 158; for the laboratory, i. 160, 161; of wort in Pasteur's flasks, i. 93
Sterilising air, i. 75, 76; definition, i. 75; discontinuous, i. 82
action of hops, i. 93
Sterility, relative, i. 93
Stichococcus bacillaris, ii. 34
major, i. 103
Stilton cheese, action of mould, i. 247
Stimulus, ii. 43
Stock 2 (Will), description, ii. 203
Stocks 6 and 7 (Will), descriptions, ii. 203
Stockfish, dried, i. 164; reddening of, i. 109
Stracchino (Gorgonzola) cheese, action of mould covering, i. 247; conversion of albumen into fat, i. 244; nitrogen content of, i. 244
Strawberries, ii. 324
Streak culture, i. 103
Streptococcus, i. 26,* 42
acidi lactici, i. 172
erysipelatis, effect of sunlight on, i. 62
hollandicus, cause of ropy milk, i. 216; in Edam cheese, i. 216, 247
Streptothrix, i. 27, 197
Foersteri, arthrospores, i. 51
Strontium, ii. 32, 33
Strychnos leiosepala, ii. 239
Stysanus otemonitis, ii. 281
Sublimate, i. 35, 135, ii. 115
Succinate of ammonia. *See* Ammonium succinate
Succinic acid, i. 137, 240, ii. 61, 125, 150, 293, 365, 367, 368, 369; action of, on yeasts, ii. 186; produced by Allescheria Gayoni, ii. 272; Mycoderma, ii. 310; Saccharomyces apiculatus, ii. 323, 324; yeast cells, ii. 180; during fermentation of madder, ii. 342

Sucrase, ii. 384
Sugar, i. 124, 143, ii. 268, 269, 328, 340, 342, 349, 362, 364, 365, 366, 369, 370, 371, 378, 400, 401; in preventing liquefaction of gelatin, ii. 273, 274; action of alcoholase on, ii. 352; of Lactomyces on, ii. 262; of yeast on, ii. 404; action on alcoholase, ii. 352; on fermentation, ii. 350, 351, 352; as a source of carbon for yeast, ii. 150; consumed by yeast in relation to supply of oxygen, ii. 170; decomposition of, ii. 311, 312, 360; fermentation of, ii. 272, 289; fission of, ii. 345, 348; formation, ii. 123; fungus, i. 13; influence of strength of solution on reproduction of yeasts, ii. 167; influence on Torulaceæ, ii. 296; part played in sugar factories, i. 208; sulphur in, ii. 146
Sugar beet as a nutrient medium, ii. 215
Sugar-gelatin, ii. 374
Sugars, decomposed by Mycoderma, ii. 311, 312; fermentable and non-fermentable, ii. 381, 382; production of acids from, ii. 263, 267
Sulphate of ammonium. *See* Ammonium sulphate
Sulphates, in yeast culture, ii. 36, 146; reduction to sulphides, i. 225; retard motion of bacteria, i. 32
Sulphide of iron, a cause of blue cheese, i. 117
Sulphides, formed from sulphates, i. 225
Sulphite, ii. 36
Sulphites, action of philothion on, ii. 416
Sulphur, ii. 345; action of philothion on, ii. 415; circulation of, i. 287; importance of, in growth of fungi, ii. 36
bacteria, morphology, i. 283–287
dioxide, ii. 329; action on Saccharomyces apiculatus, ii. 320, 322, 329; influence on yeasts, ii. 295
Sulphurea, ii. 36
Sulphuretted hydrogen, i. 225, 284-286, ii. 128, 147, 275, 345, 381; liberated by Penicillium, ii. 275; produced by yeast, ii. 174, 415, 416
Sulphuric acid, ii. 387, 390, 392, 395; action of, on micro-organisms, ii. 179; on proteolysis, ii. 411; produced by yeast, ii. 412
Sulphurous acid, i. 84, 85, 174, ii. 416
Sumach infusion, micro-organisms, i. 207
Sunlight, action on Saccharomyces apiculatus, ii. 320; on yeasts, ii. 42; decomposing power of, i. 20; protective effect of pigment, ii. 43
Superphosphate for preventing loss of nitrogen, i. 257
Suspensor, ii. 13
Sweet ensilage, i. 200–202
rot, ii. 73
Symbiosis, definition, i. 66
Sympodial branching, ii. 51
Symptomatic anthrax bacilli, culture with Mic. acidi lactici, i. 67, 140; chemical action, i. 140, 178; pleomorphism, i. 70, 71
System of bacteria, Cohn's, i. 69, 70; De Bary and Hueppe's, i. 71, 72; W. Migula's, i. 72; Van Tieghem's, i. 72
Systematic grouping of bacteria, importance of spore-germination in, i. 55

Tache jaune, ii. 238
Tættegræs, i. 216
Tættemælk, i. 216
Tagatose, behaviour of Mucor Rouxii with, ii. 65
Tan liquor, ropiness in, i. 219
Tan-pit, i. 205
Tannase, ii. 271
Tannic acid as a nutrient medium, ii. 238
Tannin, i. 207, ii. 47, 181, 312, 322; effect on reproduction of yeasts, ii. 295; fermentation of, ii. 271
Tanning, i. 204–207
Tao Yu, i. 248
Taohu, i. 248
Taphrina pruni, ii. 74
Tartaric acid, i. 174, 239, ii. 276, 297, 387, 390, 392; action of Mycoderma on, ii. 310; action of Penicillium glaucum on, ii. 57; on Saccharomyces apiculatus, ii. 319; action of, on yeasts, ii. 180; as glycogen-former, ii. 125; as nutriment for Torulaceæ, ii. 295; decomposition by sunlight, i. 20; effect on a mould fungus, ii. 117; isomeric forms, i. 174; purification of yeast, i. 222
Tartaric acid, dextro-, i. 174, 176, ii. 150
lævo-, i. 174, 176, ii. 150
meso-, i. 174, 176
Tartrate of ammonium. *See* Ammonium tartrate
(dextro-) of ammonium. *See* Ammonium dextro-tartrate
(dextro-) of potassium. *See* Potassium dextro-tartrate
Tas Yu, ii. 228
Tawing, alum- or white-, i. 205; oil-, 205
Temperance ale, ii. 103
Temperature, effect of, on alcohol-fermentation, ii. 365, 366, 370; on budding of yeasts, ii. 205, 206, 207, 212, 213, 215; on cell form and development, ii. 210; on film-formation, ii. 203, 204, 205, 213, 214; on reproduction in yeasts, ii. 187; on Saccharomycetes apiculatus, ii. 320; on sporulation, ii. 203, 204, 205, 206, 207, 208, 210, 211, 212, 213, 214, 215; on Torula, ii. 302; on yeast juice, ii. 347; influence of, on bacteria, i. 28, 58–60; on budding of cells, ii. 165, 193–194, 210, 211; on Dematium pullulans, ii. 283; on endotryptase, ii. 409, 410; on fermentation, ii. 176; on formation of acids by fungi, ii. 263; on invertase, ii. 386, 387; on maltase, ii. 389, 390; on melibiase, ii. 393; on Monilia candida, ii. 331; on Oidium lactis, ii. 338; on period of generation, ii. 165, 166; on species of Aspergillus, ii. 227, 232, 234, 238, 240

242; on species of Penicillium, ii. 244, 248, 252, 253, 254; on sporulation, ii. 94, 192, 193, 194; on substances secreted by yeast, ii. 158; on top- and bottom-yeasts, ii. 194, 195
Tenacity of life by spores, ii. 21
Terfeziaceæ, affinity, ii. 218
Terpene, ii. 381
hydrate, ii. 381
Thallophyta, i. 22, ii. 3
Thallophytes, ii. 1
Thallus, i. 22, ii. 1
Thamnidium, sporangia, ii. 49
aurantiacum, sporangia, ii. 41
elegans, conidia, ii. 41; sporangia, ii. 50*
vulgare, ii. 26
Thelebolea, ii. 11
Thermogenic bacteria, i. 127, 199
Thielavia, description, ii. 219
Thiocapsa, i. 282
Thiocystis, i. 282
Thiodictyon, i. 282
Thiopedia, i. 282
Thiopolycoccus, i. 282
Thiosarcina, i. 282
Thiospirillum, i. 282
Thiothrix, i. 27; morphology, i. 280; sheath, i. 280; species, i. 280; sulphur content, i. 280
nivea, morphology, i. 280*
tenuis, i. 280
tenuissima, i. 280
Thread fungi, ii. 6
Thrush disease, ii. 334
Thymene, ii. 387
Thymin, ii. 119, 120
Thymol, ii. 390, 408, 409, 410
Thymol-gelatin, preparation of, for enzyme-testing, i. 231
Thymus gland, ii. 119
Tibi, ii. 213
Tilletia, conidiospore, ii. 80
Tin culture vessels, ii. 31
Tinned meat, i. 167, 168
Tobacco, ii. 288; as a culture medium, ii. 232; fermentation, i. 128, 238, ii. 62
Tofu, i. 248
Tokelau disease, ii. 235
Toluene, ii. 387, 391, 394; action of, on yeasts, ii. 181; action on fermentation, ii. 350, 357
Toluol, ii. 409, 410, 416
Top-fermentation, definition, ii. 82; distinction from bottom-fermentation, ii. 133, 134
Top-fermentation beer yeasts, ii. 99; convertibility into bottom-fermentation forms, ii. 90; gum from, ii. 128, 129; pentosans, ii. 129
yeast cells obtained from bottom yeast, ii. 194, 195
Top-yeasts, ii. 203, 204
Torula, i. 170, ii. 7, 20, 286, 330, 332; acids produced by, ii. 297; action of sunshine on, ii. 42; affinities, ii. 213; alcohol as food for, ii. 295; alcoholic fermentation ii. 7; ascospores, ii. 78; beer as food for, ii. 294; budding, ii. 7, 8*; catalase in, ii. 297; cell forms, ii. 286, 287; effect of desiccation on, ii. 297; effect of temperature on, ii. 295, 297; granules, ii. 113; habitat, ii. 185, 186; maladies in beer and wine due to, ii. 299; philothion in, ii. 416; "red Torula," ii. 299; use in preparing foodstuffs, ii. 298; vacuole enclosures, ii. 111
Torula amara, ii. 289, 294, 297
cerevisiæ, ii. 202, 289
colliculosa, ii. 293, 295, 296, 384, 389, 394
Duclauxii, ii. 289, 295
lactis, ii. 289
monilioides, ii. 289
nigra, ii. 302
Novæ Carlsbergiæ, ii. 294, 296, 297
pulcherrima, ii. 292
tyrocola, ii. 289
Torulaceæ, ii. 286–302, 384; acids produced by, ii. 297; colour in, ii. 294; delimitation, ii. 286–290; distribution, ii. 290; effect of dense liquids, ii. 294, 295; of media, ii. 295; of temperature, ii. 295; of oxygen, ii. 295, 296; and of sugars, ii. 296; enzymes produced by, ii. 296, 297; fats in, ii. 292; fermentative power in, ii. 296; film-formation by, ii. 294; longevity in, ii. 298; maladies in beer and wine produced by, ii. 298; nutrient media for, ii. 293, 294; oil in, ii. 292; pathogeny of, ii. 298; variation, ii. 290
Torulaspora, ii. 201; description, ii. 209
Delbrückii, ii. 209, 287
Toxicity explained, i. 91
Toxins, synthesis of, i. 96
Tradescantia discolor, ii. 45
Transverse division, i. 42
Traube's theory of fermentation, i. 17, ii. 340
Trees, exudation from, ii. 100
Trehalase, ii. 396; secreted by Allescheria Gayoni, ii. 269; by Aspergillus niger, ii. 268; by Penicillium glaucum, ii. 269
Trehalose, ii. 296, 396, 397, 406; behaviour of γ-Amylomyces with, ii. 66; of Mucor Rouxii with, ii. 65; fermentation by Mucor alternans, ii. 61; fermentation of, ii. 332
Tricarballylic acid, behaviour of Bacillus typhi abdominalis towards, i. 241
Trichophytis, ii. 235
Trichothecium resin, chitin in, ii. 28; effect of light on, ii. 41
Trifolium, nodule-formation, i. 265
repens, nodule-formation, i. 268
Trimethylamine, i. 106, 128, ii. 380, 413
True branching, ii. 2
Trypsin, i. 230, ii. 46, 118; in asporogenic cells, ii. 191
Tryptase, ii. 413
Tryptophane, ii. 274
Tuber æstivum, lignification, ii. 30

Tuber cibarium, ligulification, ii. 30
Tubercle bacillus, differential colouring, i. 51; in milk, i. 155; microscopical range, i. 155, 156; mortal temperature, i. 156; on grapes in the Vienna market, i. 168; resistance to salt, i. 165
Tuberculosis of cow, i. 155; of goats, i. 156
Tubular buds, ii. 2, 3, 300, 301
Turanose, ii. 396, 399; from melecitose, ii. 268
Turnip flavour of butter, i. 182
Typhus bacillus. *See* Bacillus typhi abdominalis
Tyrosine, ii. 274, 345, 380; decomposition of, by Bac. mycoides, i. 235; formation of, ii. 46; formed in fermentation of Soja bean, i. 247; metabolic product of species of Tyrothrix, i. 245; occurrence in beet, i. 310; origin and restriction of, i. 187, 224, 225; produced by yeast, ii. 121, 412
Tyrosinase, i. 310, 311
Tyrothrix catenula, i. 245
claviformis, i. 245
distortus, i. 245
Tyrothrix filiformis, i. 245
geniculatus, casease, i. 245; cause of bitter butter and milk, i. 252; lab-secretion, i. 245; metabolic products, i. 245
scaber, action of light on, i. 60, 61; casease, i. 245; lab-secretion, i. 245; metabolic products, i. 245
tenuis, i. 245
turgidus, i. 245
urocephalum, i. 245
Tyrotoxicon, cheese poison, i. 233
Tyrotoxin, i. 233

Ulothrix subtilis, behaviour in absence of calcium, ii. 34
Ultra-red rays, effect on Coprinus niveus, ii. 40
Ulvina aceti (Bac. aceti), i. 23, 295
Unhairing, i. 204
Universal nutrient medium, i. 96
Unorganised ferment, i. 17
Uracyl, ii. 119, 120
Uracyl, 5-methyl-, ii. 119, 120
Urase, ii. 275, 342; behaviour of higher plants to, i. 254; decomposition of, i. 254; fermentation (enzyme), i. 18; influence of air on the separation of, i. 78; nature and properties, i. 18, 257; power of fermentation, i. 255; production of, by Bacterium ureæ, i. 255; by Micrococcus ureæ liquefaciens, i. 255; by Urobacillus Pasteurii, ii. 255, 256; by U. Freudenreichii and U. Schützenbergii, i. 256; rapidity of fermentation, i. 255
Urea, i. 253 *et seq.*; artificial formation of, i. 14, ii. 150, 412; as a source of nitrogen for yeast, ii. 156; fermentation by Proteus vulgaris, i. 228
Uredineæ, ii. 17; cellulose content, ii. 26
Urethane, ii. 275
Uric acid, ii. 119, 275; decomposition and fermentation of, i. 257
Urine, action of light on, i. 61; ammoniacal fermentation of, i. 257; bath, i. 120; nitrogen loss caused by bacteria, i. 254; test for arsenic in, ii. 38; viscid, i. 219
Urobacillus, i. 255; Freudenreichii, i. 256; Pasteurii, i. 255, 256; Schützenbergii, i. 256
Urobacteria, Miquel's genera, i. 255, 256; action of acid on, i. 256; of ammonium carbonate on, i. 256; occurrence of, i. 256, 257
Urocephalum, i. 47
Urococcus, i. 255
Urosarcina, i. 255
Usnea barbata, ii. 26
Usnein, ii. 26
Ustilagineæ, ii. 17, 288; cellulose content, ii. 26; conidiophores, ii. 80
Ustilago ficuum, ii. 239
phœnicis, ii. 239

Vaccinum vitis idæa, i. 91, 108
Vacherin cheese, i. 243, 244
Vacuole, ii. 109; enclosures, ii. 112
Vacuoles, ii. 331, 332
Valerianic acid, ii. 150, 378; formed by film yeast cells, ii. 92
Valeric acid, ii. 276, 372
Variability of Saccharomycetes (yeast cells), ii. 90, 91, 189, 198
Varnish for preservation of eggs, i. 167
Vascular cryptogams, i. 22
Vaucheria, calcium need, ii. 34
Vegetables as a culture medium, ii. 234; compressed, i. 168; desiccated, i. 167; preserved, i. 167
Velocity of reproduction. *See* Reproductive power
Vesicular bacteroids, i. 269
Vibration, influence of, on bacteria, i. 63–65
Vibrio, generic name, i. 26, 69; in Cohn's classification, i. 70; in mucin, i. 26
choleræ asiaticæ, diastase-formation, i. 148; effect of H_2O_2, i. 86; effect of light, i. 62; of lithium, i. 36; of milk of lime, i. 87; form of liquefied funnel, i. 103; indole reaction, i. 224; occurrence in eggs, i. 167; period of generation, i. 44; production of lævolactic acid, i. 179; proteolytic enzyme, i. 230; splitting of fat, i. 153
cyanogenus. *See* Bacterium syncyaneum
Finkler-Prior, proteolytic enzyme, i. 230
lineola, i. 226
rugula, i. 29,* 47*
synxanthus. *See* Bac. synxanthum
Vibrion butyrique, i. 143, 149
septique. *See* Bacillus œdematis maligni
Vicia faba, nodule-formation, i. 262,* 263; reticular band of bacteroids, i. 267
sativa, development of bacteroids, i. 267*
Vienna process of making pressed yeast, ii. 82

Vin filant, i. 217; huileux, ii. 217; mildiouse, i. 241; tourné, i. 193, 241
Vine, disease in, ii. 278
Vinegar eels, i. 307
flowers. *See* Mother of vinegar
fly, i. 305
Vinegar manufacture, i. 302–307; manufacture of pure culture ferment, i. 305–307; preservation, i. 11
plant, i. 295–298
Vino filante, i. 217; girato, i. 239
Virulence, i. 55, 172
Viscose, i. 212
Viscosin, ii. 29
Viscosity due to Dematium pullulans, ii. 283
Vitaceæ, ii. 262
Vitalistic theory of Cagniard-Latour, i. 11

Wädensweiler cyder yeast, ii. 133
Wall saltpetre, i. 89, 293
Wall-paper, arsenic in, ii. 37
Warmth-loving bacteria, i. 59
Water, ii. 362, 363, 375; derived from alcohol, by Mycoderma, ii. 311; from sugar, by Mycoderma, ii. 312; produced by fermentation, ii. 362, 363; by Willia anomala, ii. 214
Water-bacillus, analysis of dry matter, i. 35
Water-bacteria in brewery, i. 102; in subsoil, i. 137
Water-filter, efficiency of, controlled by hydrogen peroxide, i. 86
Water-retting, i. 152
Welwitschia mirabilis, ii. 239
Wheat, ii. 383; pentosans in, ii. 151
White beer, i. 195
Whortleberries, i. 108
Whortleberry, benzoic acid in, i. 91
Willia, ii. 291, 293, 303, 403, 413; definition, ii. 202; description, ii. 213; sporulation experiments with, ii. 192
anomala, description, ii. 213, 214
anomala I., description, ii. 214
anomala II., description, ii. 214
anomala III., description, ii. 214, 215
belgica, ii. 403; description, ii. 215
Saturnus, description, ii. 215
Wine, ii. 367, 371, 372, 375, 379, 380, 401, 413; action of oxydases on, i. 308; Torulaceæ on, ii. 298; air taste, i. 308; bacteria in, i. 194; bittering of, i. 311; black break, i. 193; braunwerden, i. 309; break, ii. 135; browning of, i. 309; fat in, ii. 127; lactic fermentation, i. 194; loss of colour in, i. 239; pasteurisation, i. 194, ii. 103; putrefactive fermentation, i. 239, 240; Rahn-werden, i. 309; removal of acids, i. 240; ropiness in, i. 217; ropy, ii. 129; sugar in, ii. 147; sulphur dioxide in, ii. 147; versieden, i. 239; vinous fermentation according to Schwann, i. 13; white break, i. 193; Zickend-werden, i. 193
Wine deposit, composition, ii. 87; fat in, ii. 126
Wine must as food, i. 95, ii. 295; concentrated, i. 169; preservation of, i. 169
Wine yeast, fission of cell in fluid cultures, ii. 96; Johannisberg I., ii. 81; Rauenthal, ii. 82; sporulation, ii. 97 *et seq.*; sulphuretted hydrogen in, ii. 147; winter in soil, i. 138
Wine-filter, i. 79
Wine-making, action of Saccharomyces apiculatus in, ii. 324
Winningen wine yeast, salt needed by, ii. 32
Woollen fabrics as a culture medium, ii. 234
Wort, as a culture medium, ii. 168, 169, 170, 171, 189, 190, 192, 196, 197, 228; ropiness in, i. 219; sterilising, i. 93
Wort-gelatin, ii. 160, 162, 163; as a culture medium, ii. 189, 196, 212, 213, 214, 217, 232; preparation, i. 100
Wound pus, white, i. 62; yellow, i. 62
Wuck, ii. 344
Wurzel bacillus. *See* Bacillus ramosus

Xanthin, i. 35, 247, ii. 119, 120, 121, 275, 345, 412
Xanthoprotein reaction, i. 35
Xylene, action of, on yeasts, i. 106, ii. 181
Xylose, ii. 382; behaviour of Mucor Rouxii with, ii. 65

Yeast, ii. 389; acetic bacteria and, ii. 78; action on gastric juice, ii. 113, 114; analysis of stock, ii. 98; articulated mycelium, ii. 92; artificial, ii. 69; artificial souring, ii. 181, 182; as a coffee substitute, ii. 122; ascospores, ii. 96; ash content, ii. 139–142; ash reaction, ii. 140; autofermentation, ii. 404–407; break, ii. 135–138; carbon obtained from carbohydrates by, ii. 150; Carlsberg bottom yeasts No. 1 and No. 2, ii. 86; cell nucleus, ii. 109–112; cell-division, ii. 111; cellulose in, ii. 107; chemical behaviour of the cell membrane, ii. 106–107; chemistry of, ii. 117; Chinese, ii. 63, 67; chitin in, ii. 107; cholesterin in, ii. 127; conidia, ii. 16, 77; conversion into lactic acid bacteria, ii. 78; cultivation and reproduction, ii. 139, 159–164; definition, ii. 79–81; dextran, ii. 128; discovery by Schwann, i. 13; effect of amount of sowing on total crop, ii. 168, 169; effect of amount of supply of oxygen on respiration and reproduction, ii. 169–172; effect of cold, ii. 22; of copper salts, ii. 173–175; of light, ii. 39; of sunshine, ii. 42; on dextrin, ii. 401; on raffinase, ii. 398; on trehalose, ii. 396, 397; fat content, ii. 126, 127; film-formation, ii. 90–93; fission, ii. 75; Frohberg, ii. 81; gelatinous network, ii. 130,* 131; glycogen in, ii. 123–126; granules, ii. 112–115; head, ii. 132; hydrolytic enzyme, ii. 124; importance of phosphoric acid, ii. 143–146; of sulphur, ii. 146, 147; of sulphur dioxide, ii. 146, 147; improvement

in the industry, i. 191–193; influence of potash, ii. 139; involution forms, ii. 87; keeping, ii. 162; lecithin in, ii. 127; macrochemical examination, ii. 108; mannan, ii. 128; mineral foodstuffs, morphology and life-history, ii. 72; mould yeast, ii. 90; names, i. 12; nitrogen from asparagin, urea, peptone, ii. 154, 155; worts, musts, and wines, ii. 157, 158; nitrogenous constituents, ii. 117 *et seq.*; pentosans, ii. 129; permanent cells, ii. 91,* 92,* 112,* 113*; preparation of media, ii. 117; pitching, i. 188; utilisation of alcohol, ii. 151; pure cultures with borax, ii. 131; putrefaction by, i. 232; racking, ii. 137; Saaz, ii. 81; spherical, ii. 7; sulphuretted hydrogen formation, ii. 147; testing apparatus, ii. 98, 99; turbid beer, ii. 136

Yeast cells, action of, on sugars, ii. 385; beer, ii. 149; behaviour of, towards alcohol, ii. 175–178; bottom, ii. 149; chemistry of, ii. 117–138; cultures, ii. 89; influence of carbon dioxide on, ii. 178, 179; reproduction of, ii. 159–172; secretion of invertase by, ii. 384–388; specific gravity, ii. 148; spores, ii. 19; starch in, ii. 148, 149; top, composition, ii. 149; use of carbon compounds, ii. 149, 150; variations, ii. 89 *et seq.*

Yeast counter, ii. 136
enzymes, ii. 340–358
glucase, ii. 389
gum, ii. 118, 128
(Jopen), No. 602, ii. 397
juice, action of agents on, ii. 347–352; fermentation of, ii. 363, 371; preparation of, ii. 342–344
(Kretschmer beer), ii. 397
Küster Tokay, No. 534, ii. 393
maltase, ii. 390
mash, i. 189, ii. 71
No. 2, Victoria, ii. 393
Yeast No. 54, Dürkheim, ii. 393
No. 389, Gräfenthal, ii. 393, 394
No. 600, Jopen beer, ii. 393, 394
No. 603, Jopen beer, ii. 393, 394
ring, i. 93
samples, carriage of, ii. 159
sugar, ii. 123
vacuoles, ii. 111
water, ii. 94, 95; influence on reproductive capacity of yeasts, ii. 166, 167; influence on reproduction of Torulaceæ, ii. 295; preparation of, ii. 121
wine, ii. 131
zymase, ii. 345, 349
Yeasts, action of acetic, oxalic, formic, succinic, malic, and citric acids on, ii. 180; of organic stimulants and poisons on, ii. 180–182; behaviour of, towards hop resins, ii. 180, 181

Zinc, ii. 33
chloride, action of, on yeasts, ii. 179
lactate, i. 173
sulphate, action of, on yeast, ii. 179
Zomerbier, i. 195
Zooglœa, ii. 1; formation, i. 31, 32, 208
Zoospore, ii. 12
Zygomycetes, characteristic, ii. 12; fermentation, ii. 48; in the fermentation industry, ii. 14
Zygosaccharomyces, affinities, ii. 209; definition, ii. 201
Barkeri, description, ii. 209, 210
priorianus, description, ii. 210
Zygospores, ii. 11, 48; formation, ii. 13–15; suspensor in Sporodinia, ii. 50
Zygote. *See* Zygospores
Zymase, ii. 343, 345, 352–356
Zymin, ii. 353, 407
Zymogen, ii. 358
Zymogenic bacteria, i. 73; nitrogenous nutriment, i. 96

Printed by Ballantyne & Company Ltd
Tavistock Street, Covent Garden, London

DATE DUE

Unless this book is returned on or before the last date stamped below a fine will be charged. Fairness to other borrowers makes enforcement of this rule necessary.

3 '79				
DE 12 '85				
MAY 10 1990				

Misericordia University
3 1999 00031 2245